

# PHILOSOPHICAL TRANSACTIONS.

## I. *On the Gastric Gland of Mollusca and Decapod Crustacea: its Structure and Functions.*

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[PLATES 1-4.]

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### *Preliminary.*

IN 1883 I communicated a paper to the Royal Society,\* in which I described the occurrence of a pigment closely resembling vegetable Chlorophyll in the so-called "Liver" of Invertebrates; and in 1885 a further contribution in continuation of the same subject, which was published in the 'Philosophical Transactions' (Part I., 1886).

\* 'Roy. Soc. Proc.' vol. 35 (1883), p. 370.

This colouring matter I named from its likeness to Chlorophyll, Entero-chlorophyll. I showed in the latter paper that it occurred in the glandular epithelium lining the liver tubes, either in granules, or dissolved in oil globules, or in a more or less diffused form, in the latter case staining the cell-protoplasm.

I have not been able until lately to follow up this subject. Meanwhile, a number of observations bearing on the structure of this gland have been published, and other observations more or less bearing on the nature of its secretion.

As some recent writers have questioned the right of calling the pigment by the above name, viz., Entero-chlorophyll, I have made further observations to enable me to decide whether I was justified in my conclusions or not.

To make the inquiry complete I had to study the minute structure of the gland and the nature of the contents of its glandular epithelium. Previously, indeed, I attempted to do this, but it was found to be no easy task, owing to the difficulty of fixing, hardening, and cutting sections, a difficulty which has been encountered by other observers. However, I have now succeeded in getting some very good sections not only showing the structure of the gland, but also preserving the exact shape and natural colour of the cell-inclusions.

*Historical.*—One does not find much about the structure of the gastric gland in the text-books of Zoology or Comparative Anatomy. In HATCHETT JACKSON'S edition of ROLLESTON'S "Forms of Animal Life," and in LANG'S "Text-book of Comparative Anatomy," a brief *résumé* of recent observations is, however, given.

Three investigators have, within recent years, enlarged our knowledge, and to them we owe an exact description, of the histology of this gland, and of the micro-chemical reactions of its cell-inclusions, namely, MAX WEBER,\* DIETRICH BARFURTH,† and JOHANNES FRENZEL.‡ These writers give full references to all the important literature of the subject.

One of the most satisfactory accounts of the structure in general of the gastric gland, at that time known as the Liver (Leber) of Invertebrates, is to be found in FR. LEYDIG'S "Lehrbuch der Histologie des Menschen und der Thiere." LEYDIG had previously studied the structure of the gland in his beautiful treatise on *Paludina vivipara*,§ and subsequently, though more briefly, in another treatise on *Cyclas cornea*.|| LEYDIG showed that, where among the invertebrate animals a liver occurs as an independent organ, separated from the gut, as among Crabs, Spiders, and Mollusca, it always consists of a connective tissue basis, the *tunica propria*, and of secreting

\* WEBER, "Ueber den Bau und die Thätigkeit der sog. Leber der Crustaceen," 'Archiv f. Mikroskop. Anatomie,' vol. 17 (Bonn, 1880).

† BARFURTH, "Ueber den Bau und die Thätigkeit der Gasteropodenleber," *ibid.*, vol. 22, 1882.

‡ FRENZEL, "Ueber die Mitteldarmdrüse der Crustaceen," 'Mittheil. a. d. Zoolog. Station zu Neapel,' 1883, Hft. 1; also "Mikrographie der Mitteldarmdrüse (Leber) der Mollusken," 'Nova Acta der Ksl. Leop.-Carol. Deutschen Akademie der Naturforscher.,' vol. 48 (1886) and vol. 60, 1893.

§ "Ueber *Paludina vivipara*, &c.," 'Zeitschrift für wissen. Zoologie,' vol. 2, 1850, p. 125, &c.

|| "Ueber *Cyclas Cornea* (Lam.)," 'MÜLLER'S Archiv,' 1855, p. 47, &c.

cells. In general there are two kinds of glands, namely, the "Blind-sack-förmigen," which I suppose one may translate as closed cæcal (or tubular), and the spongy or cavernous type. The liver, for example, is either represented by a few simple, short unbranched closed tubes (Entomostraca, Phyllopods), or the few "Blindsäcke" are long pouches ("Schlauche," *e.g.*, Isopods, Amphipods, and among the Mollusca, *Creseis*), or they are ramified (*Argulus* among Mollusca), and are numerous among Cirripedia and higher Crustacea.

The bivalve mollusca have a similar folliculose liver, so also many Gastropods and Heteropods, *e.g.*, *Ostrea*, *Cyclas*, *Dreissena*, where the follicles are short, and *Unio* and *Anodonta*, where they are longer. In those cases where the follicles divide up frequently, and anastomose, we find the common type of liver which comes to resemble the liver of vertebrates. Such a kind of liver is found in *Limax*, *Paludina vivipara*, and other Gastropods, and even more pronounced in *Tethys*, *Doris*, *Tritonia*, &c. *Squilla* seems also to possess a cavernous liver.

LEYDIG then goes on to say that it is hardly necessary to remark that between the simple follicular and the common liver type transitional forms must occur.

The *tunica propria* he describes as a mostly quite homogeneous covering (Haut), and shows that sometimes muscular tissue is found outside this layer, for instance, in *Paludina* on the peritoneal surface of the organ, as well as between its follicles; and surrounding the liver follicles in many Crustacea, *e.g.*, *Oniscus*, *Gammarus*. MAX WEBER\* figured these subsequently, and described their functions in the Isopods and Amphipods, where they form very complicated structures.

Outside of these two layers—the *tunica propria* and *tunica muscularis*—another connective tissue layer of a harder consistence may be found, which is the analogue of the *tunica serosa* of the gut (LEYDIG). Subsequent observers, such as MAX WEBER and BARFURTH, have tried to trace these three layers, the former in certain Crustacea, the latter in Gastropod Mollusca; but I am unable to trace all of these in the latter, or in Decapod Crustacea. Of course the *tunica propria* always can be recognised, and directly to it—as all observers are agreed—the gland cells are attached.

LEYDIG further describes those "secretion cells" which lie on the inner surface of the *tunica propria*. These are ciliated in the *ductus hepaticus* of the Mollusca, but this ciliation seldom extends to the end follicles of the gland (in them). *Cyclas*, and perhaps Cephalopods, are an exception however. The peculiar secreting cells of the liver of all other Mollusca are (he says) without cilia. As to the contents of those cells, LEYDIG says they are very like those of the Vertebrates, being either pale granular masses, or yellow-brown coloured grains. In embryonic livers of *Cyclas* he saw, at the end "des Eilebens," some of the cells filled with fluid yellow-coloured contents and many yellow crystals. In FRENZEL's treatises referred to above, such crystalline contents are figured in the gland cells of many Mollusca and Crustacea;

\* *Loc. cit.*

but I shall have to refer to these crystals further on. So far as I can see LEYDIG does not describe the ciliation of the gland cells of Crustacea which is in many very well marked, as is well known.

It is hardly necessary to refer to the writings of observers earlier than those of LEYDIG, so I may merely mention the names of JOHANNES MÜLLER (1830), SCHLEMM (1844), KARSTEN (1845), MECKEL (1846), and WILL (1848). Then came LEYDIG's treatise already mentioned, followed by those of LEUCKART (1854), of GEGENBAUR (1855), and then LEYDIG's beautiful "Lehrbuch" followed in 1857. We next have SEMPER's\* "Contributions to the Anatomy and Physiology of the Pulmonates" in 1857, and CLAPAREDE's† paper on *Neritina fluviatilis* in the same year.

The same author in his paper on *Cyclas elegans*‡ (published the year after), described three components among the liver cells, (1) solid brown bodies (Klumpen: from 0.015 to 0.04 mm.), of which each occupied a cell; (2) colourless cells filled with pale granules, and (3) fat-like grains or drops, but which were not fat. At the same time LACAZE-DUTHIERS§ treatise, "Histoire de l'Organisation et du Développement du Dentale," appeared, followed by another treatise on the same,|| in which he described the liver cells, of which he found only *one* kind. In *Pleurobranchus* he subsequently¶ found two kinds of cells, "hepatic" cells, whose contents were globular and granular, strongly coloured; and other cells filled with small brown bodies. He also found ovoid corpuscles of a blue-violet colour, which developed gas with acids, and which he, therefore, took for lime.

HESSLING\*\* in 1859 also describes in his treatise "Die Perlmuschel und ihre Perlen," &c., different kinds of cells from this animal's gastric gland. Some contain pale granular masses, others a yellow-brown pigment, which is partly diffuse or partly in the form of large grains. These are evidently the two kinds of gland cells of later observers, namely, the so-called liver-cells (BARFURTH) or granular cells (FRENZEL), and the so-called ferment cells. VOIT†† in 1860 showed by chemical proofs that neither sugar (which had been found by CLAUDE BERNARD‡‡ in the gastric gland of Mollusca previously) nor bile-acids, nor bile pigment, can be shown by chemical proofs to be present in the liver of the animal examined by HESSLING.

H. SICARD§§ in 1874 described two kinds of gland cells in *Zonites algirus*, but he did not sufficiently distinguish them from each other.

\* SEMPER, 'Zeitschrift für wiss. Zoologie,' vol. 8, 1857, p. 340, &c.

† CLAPAREDE, 'MÜLLER's Archiv,' 1857, p. 109, &c.

‡ CLAPAREDE, 'MÜLLER's Archiv,' 1858, p. 1, &c.

§ 'Annales des Sciences Naturelles,' série 4, Zoologie, t. 6.

|| Paris, 1858.

¶ 'Annales des Sciences Naturelles,' série 4, Zoologie, t. 11, 1859.

\*\* HESSLING, Leipzig, 1859.

†† VOIT, 'Zeitschrift für wiss. Zoologie,' vol. 10, 1860, p. 470, &c.

‡‡ CLAUDE BERNARD, 'Annales des Sciences Naturelles,' série 3, t. 19, 1853, p. 282, &c.

§§ 'Annales des Sciences Naturelles,' série 6, t. 1, 1874.

Passing over the names of others who have written on the Molluscan or Crustacean gastric gland, I may say the first who really accurately described the organ in Crustacea was MAX WEBER.\* He, however, limited his observations to Isopods, Amphipods, and Decapods. After describing in the most accurate manner the general structure of the gland and its "tunics"—*propria*, *muscularis*, and *serosa*—he comes to the conclusion that the gland cells are of two kinds: (1) liver cells, and (2) ferment cells. By the use of osmic acid he was led to conclude that the one kind of cell, the ferment cell, became at once deep black; the other, the liver cell, darkened after longer action. WEBER found no glycogen in the gland, and he thought he had proved satisfactorily that the ferment cell formed the ferment, the other cell, the liver cell, was the place of formation of pigments and other "separation products." Led astray by the notion, prevailing before the time of the publication of his paper, as to the "hepatic" function of the gastric gland, WEBER thought his studies justified his conclusion that this gland is a "Hepatopancreas." It is important to note further that WEBER came to the conclusion that the pigment of the crustacean liver was combined with a fat-like body (an einen fettartigen Körper gebunden). In 1883 FRENZEL† published his valuable paper "On the Mid-gut Gland of the Crustacea" ("Über die Mitteldarmdrüse der Crustaceen"), based on observations made mostly at Naples. He evidently experienced great difficulty in preserving the epithelial gland cells, as he observes the usual fluids fail in this respect. FRENZEL came to the conclusion that WEBER was correct in calling the ferment cells by that name, but he changed the name of WEBER's liver cells to that of fat-holding (fetthaltigen) cells, or simply fat cells. According to FRENZEL and WEBER, MECKEL‡ and LEREBoullet,§ and, further, FREY and LEUCKART,|| were the first who thoroughly described the liver of Crustacea. They all found that its epithelium consisted of two kinds of cells, which MECKEL called fat- and bilin-holding cells; LEREBoullet described them as fat cells and biliary cells, while FREY and LEUCKART recognised the former as fat cells but the latter as "cells with water-clear contents." The latter authors believed that the "bile pigment" was intimately combined with the fat drops.

In PAUL MAYER's treatise on the Caprellidæ,¶ as FRENZEL indeed points out, two kinds of cells are mentioned, one kind the fat-holding cells, the other, which contains a non-fluid strongly coloured "secretion-ball" (Secret-ballen), the ferment cells. MAYER believed that in the latter cells the colouring matter of the liver secretion was prepared.

\* *Loc. cit.* He gives many references which I have omitted as being only of historical value. So also does FRENZEL, *loc. cit.*, and BARFURTH.

† *Loc. cit.*

‡ "Mikrographie einiger Drüsenapparate der Niederen Thiere," 'MÜLLER'S Archiv,' 1846.

§ 'Mém. sur la structure intime du foie,' Paris, 1853.

|| 'Lehrbuch der Anatomie der wirbellosen, Thiere,' 1847.

¶ 'Die Caprelliden des Golfes von Neapel, &c.,' Leipzig, 1882.

What these cells are like one can see by reference to Plate 4, figs. 17-21, which represent the structure of the gastric gland of the lobster. Here the so-called liver cells are filled with highly refracting fat globules, &c., while the ferment cells contain a vesicular-looking structure containing a granular mass, which is generally coloured more or less. The liver cells of the Caprellidæ were found by WEBER to contain fine greenish granules in abundance. The form and size of these latter cells in various Crustacea are accurately described by FRENZEL, also their contents. This consists of (1) the secretion, (2) protoplasm, and (3) nucleus.

The secretion, he says (speaking of Decapods), is formed of "a more or less great amount of strongly light-refracting round appearances," whose size is variable. They fill the greater part of the cell without, however, occupying the whole of it, leaving a small zone free in the upper part, and beneath the deeply-lying nucleus they are sparsely present. The granules may be coloured or colourless; and here I may remark what is of importance from my own point of view:—when they are coloured, FRENZEL shows that the colouring matter is the same as that of the ferment cells. This colouring matter is either entero-chlorophyll or a lipochrome, or both. The contents of the liver cells easily take up colouring matter, as MAYER found to be the case in the Caprellidæ and FRENZEL in Crustacea. Thus, quite colourless globules become coloured when brought in contact with the brown secretion of the gland in which they are found, and they readily take up other colouring matters also. The contents of these liver cells were found by FRENZEL soluble in ether, chloroform, and other fat solvents without leaving a residue behind. They may also contain other small granules which may be coloured, also other contents. The cell protoplasm of these liver cells shows a longitudinal striation close to the lumen of the tube (see Plate 4, figs. 19 and 20), and generally cilia projecting into the latter.

The "ferment cells" are described by WEBER and, indeed, by FREY, LEUCKART, and others) as containing a clear watery-looking vesicle ("wasserhelles Secretbläschen"). In the Caprellidæ PAUL MAYER did not find such, but a green coloured opaque spherical body (Ballen), which is *not* fluid. When freed from neighbouring cells they appeared roundish, as do also the fat-holding cells. The same remark applies to both kinds of cells in the Mollusca, as we shall see later on. When they are in apposition to other cells they vary in shape from pressure. These various shapes are described at length by FRENZEL, to whose paper I may refer as to this point. These ferment cells are fewer in number than the fat-holding cells, and they are more abundant during feeding than during fasting periods, so that they must play an important part in digestion. Correspondingly, "in fasting Decapods during hunger, or when placed in sea water for some time without food," the gastric gland becomes more transparent and less coloured. Where these cells project into the lumen of the tube they are ciliated, and the underlying parts of the cell are striated. They contain an egg-shaped or round mass, which, in the ripe condition, almost fills the containing cell. This mass is said by FRENZEL to be contained in a membranous bladder, and in

all the Decapod Crustacea is granular and coloured. This is the case not only among sea-water, but also in fresh-water forms such as *Astacus fluviatilis* and *Palæmonetes varians*. The colour of the mass in the ferment cell is a bright or dark brown, and correspondingly the gland and its secretion are coloured, or it may be a rusty-brown or bright yellow. But even in the same species the colour may vary without assignable cause. Normally, this coloured mass in the ferment cell is finely granular, and the granules may be collected into secondary spherical masses within the primary one, e.g., in *Maja*. FRENZEL describes departures from the typical appearance of these cells, which no doubt are due to the fact that we cannot draw hard and fast lines between the two kinds of cells. Indeed, as LANG in his "Comparative Anatomy" remarks: "We cannot also carry out a sharp distinction of the cells into ferment cells and hepatic cells; many transition forms occur."

FRENZEL found *crystals* in the vesicles of the ferment cells in some *Decapods*, notably in *Maja* and *Carcinus*, also in *Callinassa*, *Squilla mantis*, and *Dromia*. These crystals were colourless and needle-shaped. He believed they consisted of *tyrosin*, pointing out, as he remarks, another point of resemblance between the gastric gland of Crustacea and the vertebrate pancreas, since the latter contains tyrosin in its secretion. In one case crystals of an unknown nature were found, of a cubic shape and of a brownish colour. They occurred in *Dromia*.

FRENZEL also investigated the "mid-gut" gland of the Isopods and Amphipods, and finally concluded that among all Crustacea the secretion of the gland contains fat in coloured or colourless drops, which is formed either in special cells, as in Decapods, Gammaridæ, or Caprellidæ, or in the usual secreting cells, as in the Isopods and Phronimidæ. With the single exception of the Isopods, these cells contain, further, small round bodies which join to form a small mass.\*

The principal component of the secretion is formed mostly of very fine and coloured granules, which, in the Decapods, Gammaridæ, and Caprellidæ, are developed in special cells—the ferment cells; but in the Isopods and Phronimidæ, together with the fat, in another kind of cell. FRENZEL also found a *third* kind of cell, the young one, whose protoplasm stains more deeply than that of the others. This cell is destined to replace the others. He concludes that the mid-gut gland of Crustacea is in no sense a liver, but a pancreas. A little before this important paper of FRENZEL was published, indeed almost at the time of its publication, appeared BARFURTH'S paper "On the Gastric Gland of the Gastropod Mollusca" ("Ueber den Bau und die Thätigkeit der Gasteropoden-Leber"). In this paper BARFURTH describes three kinds of cells, namely (1) liver cells, (2) ferment cells, and (3) lime cells. The last, according to him, contain phosphate of lime, destined to repair the shell of the snail when broken, or to supply lime for the formation of the epiphragm at the beginning of hibernation. I may state at once that FRENZEL contradicts

\* I here give the sentence in German, "ferner überall kleine kugelförmige Gebilde welche zu einem Klümpchen vereinigt sind."

these statements\* and dissents from BARFURTH in many other important particulars. I may, however, here give the principal conclusions at the end of BARFURTH's paper:—

(1.) The liver of the genera *Arion* and *Helix* is a compound acinose gland, whose parenchyma is enclosed by an incomplete serosa and muscularis and by a ring of closed tunica propria.

(2.) The one-layered epithelial lining contains three kinds of cells: Ferment, Liver, and Lime Cells.

(3.) The nutrition of this organ is supplied by the Arteria hepatica, whose ultimate branches end in the connective tissue spaces. These latter communicate with the blood-sinus surrounding the liver.

(4.) The epithelial lining of the gland-ducts consists of ciliated and mucus cells, but in many places only of cylinder epithelial cells.

(5.) The liver contains a special nervous apparatus.

(6.) The ferment cells of the liver form vesicles with brown-coloured ferment-spheres; the ferment can digest in acid, neutral and alkaline solutions.

(7.) The liver cells secrete small vesicles with yellowish "crumbling" (krümeligen) contents, which is excreted with the fæces.

(8.) The lime cells contain glistening spherules of phosphate of lime.

(9.) During summer, phosphate of lime is stored up in the liver, while carbonate of lime is stored in the vessel walls, and also in the connective tissue. This lime store is applied (a) in winter by *Helix* for the formation of the epiphragm, by *Arion* probably for strengthening its skin; (b) by *Helix* for the repair of the shell, by *Arion* for supplying the excretory mucus of the skin with lime salts.

Finally, BARFURTH concludes that the liver of Gastropods performs a variety of functions, which, in the higher animals, are assigned to different organs.

In 1886 the fine paper of FRENZEL† appeared ("Mikrographie der Mitteldarmdrüse (Leber) der Mollusken"), followed in 1893 by a second part, in which FRENZEL extends his observations. In these papers he limits himself mostly to the "glandular epithelium," which is figured in very elaborate coloured plates, but he confesses his difficulties in obtaining sections of the gland itself. In fact the usual hardening agents employed by FRENZEL made the preparation so hard and brittle that he could not cut useful sections of the gland.

While accepting BARFURTH's conclusion that the "ferment cell" of that author prepares a ferment, FRENZEL changed the name to "club cells," or "club-shaped ferment cells" (Keulen-zellen, or keulenförmige Ferment-zellen), and to the liver cells of BARFURTH he gave the name of "granular" cells (Körnerzellen).

I think it is due to myself that I should here call attention to the way in which FRENZEL has misrepresented a statement of mine. In my paper published in the 'Proceedings of the Royal Society' in 1883, I said that in the liver of the snail there

\* FRENZEL, *loc. cit.*

† *Loc. cit.*



were bodies "which remind one strongly of unicellular algæ, the exact nature of which I have not yet determined;" and later on he gives me credit for mistaking the ferment cells for unicellular algæ. This is not so; saying a thing is like another thing is not the same as saying they are identical. Indeed, in a subsequent paper published in the 'Philosophical Transactions' for 1885, I showed that unicellular algæ are *not* present. However, Herr FRENZEL does not seem to have seen that paper. I shall have occasion further on to refer to FRENZEL's observations, so I need not now give further quotations from his papers, except to call attention to the fact that he does not think the colouring matter found in the glandular epithelium is related to chlorophyll. However, he has not used the spectroscope in his work, and is not, therefore, in a position either to confirm or deny the truth of my statements. He acknowledges, however, that there is no difference between the pigment colouring the contents of the ferment and the fat-holding cell of Crustacea, and that colouring the contents of the ferment cell and the granular cell of the Mollusca. And I am now quite certain that this pigment is either entero-chlorophyll, in most cases, or an associated lipochrome. In the Crustacea either of these is, however, present in much less amount than in Mollusca. It is hardly necessary here to point out that since chlorophyll is an indefinite term used in a different sense by different observers, it would be safer to restrict this name to the green or blue-green constituent of the mixture extractable from leaves by fat solvents, the spectrum of which consists, in the case of unaltered chlorophyll, of the four bands in the red half of the spectrum, or, in the case of acid chlorophyll or modified chlorophyll, of the five bands in the spectrum between Fraunhofer's lines B and F. It is in this restricted sense we must use the term chlorophyll if we wish to avoid unending controversy.

*Objects of the present Investigation.*—I was anxious to determine (1) the form in which entero-chlorophyll and the associated pigments occur in the glandular epithelium of the gastric gland; (2) the nature of the bodies with which the pigment is associated, whether, for example, the granules or other forms in which the pigment occurs are of a proteid or fatty or other material; (3) how this pigment is formed in the gland; and (4) if not actually formed there, how does it get into the glandular epithelial cells? (5) Owing to Professor LANKESTER's remarks\* on his Chætopterin, I was anxious to find out what relationship this pigment (if any) bears to entero-chlorophyll; and (6) I was anxious to study the general histology of the gland itself.

*Histological Methods.*—In studying the relationship of the pigment to the gland cells, and, indeed, in dealing with such a peculiar gland as the gastric gland is chemically, one has to adopt peculiar methods. Osmic acid and Flemming's fluid—the former so frequently used previously—were found quite useless owing to the large quantity of fat present. The very large amount of water and the small amount of

\* RAY LANKESTER, "On the Green Pigment of the Intestinal Wall of the Annelid Chætopterus," 'Quart. Journ. Micro. Sci.,' vol. 40, N.S., p. 447, &c.

coagulable material present, the extraordinary rapidity with which changes ensue in the gland, causing the destruction of its epithelial and other elements, require a rapid fixation, and yet a method of fixation which will not give rise to artefacts. I may here refer to FRENZEL'S\* method of fixing the gland of *Aplysia*, which seems somewhat heroic. He put a piece of the gland into a mixture of one part nitric acid and two parts of distilled water for about a minute, and then into a mixture of corrosive sublimate and sea water for about fifteen minutes, and after washing in water completed the hardening in different grades of alcohol. He, no doubt, succeeded in "fixing" the gland, but what about the interpretation of the appearances produced? In other cases, as a perusal of FRENZEL'S paper will show, he was as unfortunate in getting good preparations as other observers appear to have been. Hence it was that FRENZEL had to content himself with the elaborate portraiture of the epithelial cells of the gland in pictures which it will not be easy to surpass for brilliance of coloration. After various methods, which need not be referred to, and which will be found in LEE'S "Microtomist's Vade-Mecum," had been tried with little success, I finally succeeded in getting very good preparations by the following method:—

The fresh gland was fixed in formol† of from 20 to 30 per cent., not weaker, for from twelve to twenty-four hours, and then in 95 per cent. alcohol. When hard enough for cutting it was transferred to a mixture of alcohol and ether, and then into celloidin. After the celloidin had set sufficiently the mass was either cut by means of a microtome in the usual manner, or by the freezing method, as the latter allows one to cut thinner sections. In this way I was able to obtain sections which showed everything in its natural position, in which the colours of the cell-contents were preserved (as formol seems to act here as a mordant), and which are as satisfactory as possible. Of course, celloidin keeps everything in position as stated, and so allows one to see the shedding off of the epithelial lining of the gastric gland-tubes, and so on, as will be referred to further on. As stains I used Mayer's hæmalum or hæmatoxylin, or the various logwood stains, generally counterstaining with eosin; also other stains such as safranin, fuchsin, methyl blue, methylene blue, mucicarmine, "Soudan III.," thionin, &c. The sections were mounted in balsam after clearing with oil of origanum or with sandal-wood oil.

*Complete Absence of Glycogen from the Gastric Gland.*—In neither Mollusca nor Crustacea have I been able to obtain evidence of a trace of glycogen, even after applying the iodine test with all the precautions recommended by DELÉPINE.‡

I formerly showed that no evidence of the presence of bile pigments or bile acids can be obtained. Hence it is as well to drop the term "hepato-pancreas" once and for all, and also the term "hepatochromes" for its pigments, which some recent

\* *Loc. cit.*

† Containing 40 per cent. formic aldehyde.

‡ DELÉPINE, 'Proc. Physiol. Soc.,' May 10, 1891. FRENZEL came to the same conclusion, *loc. cit.*

writers would like to retain, and call the gland simply gastric gland, and when a lipochrome occurs call it by that name, or when a chlorophylloid pigment, by its proper name, entero-chlorophyll.\*

*The Gastric Gland in Lamellibranchiate Mollusca.*—We may take the gastric gland of the oyster (*Ostrea edulis*) first, as it is one of the simplest of all. I have shown its general structure in Plate 1, fig. 1. This drawing shows that here we have only one kind of secreting cell present, namely, the granular cell of FRENZEL; no ferment cells can be seen, but in the cells of the “vesicular” connective tissue, which is a very striking object in the gastric gland of the oyster, we find pigment bodies which bear a very remarkable resemblance to the contents of the ferment cells seen in the gastric gland of other Mollusca. These pigment bodies are coloured by entero-chlorophyll, and appear to be, judging from micro-chemical tests, of a proteid nature. Thus I found these spherical pigment bodies (which are shown as they appear with a Zeiss apochromatic 1/12th and compensating ocular 8 in fig. 12, Plate 3) gave the following reactions: (1) with osmic acid they seemed to become darker, though not a very distinct black; (2) with iodine in iodide of potassium they showed no marked colour change, while in the containing connective tissue cell I occasionally perceived a slightly bluish coloration; (3) I failed to dissolve out the contents of these pigment spheres with ether, alcohol, or chloroform after they had been fixed with formol; (4) with reagents for detecting cellulose, such as Schulze’s fluid and iodine followed by sulphuric acid, I could detect none; (5) the pigment spheres are stained green by methylene blue, redder than the surrounding violet-stained tissues by gentian violet; they, and indeed the gland generally, showed no trace of mucin with Mayer’s mucicarmine.† Finally, I came to the conclusion that these spherical masses of pigment are of a proteid nature, impregnated with an oily matter containing entero-chlorophyll or a lipochrome, dissolved in this oily matter. That is to say, here we have pigmented masses present in the vesicular connective tissue, which give the same micro-chemical reactions as the coloured contents of the ferment cells of other Mollusca. Some of the containing cells are fixed, being often anchored to the other cells by a long neck; others appear free, resembling in this respect ordinary wander cells. We find such pigment bodies also in the ctenidia, as indeed PECK‡ has figured them, and, perhaps, in other parts of the body of *Ostrea*, e.g., the foot. Although not shown in the drawing, we find the entero-chlorophyll present in the granular cells lining the acini of the gland, as well as in these pigment spheres. It seems to me that when pigment begins to form or to be deposited in these connective tissue cells it is preceded by a colourless, or almost colourless, substance, and then becomes yellow, and finally dark brown, or greenish-brown. Correspondingly, the pigment sphere is at

\* I once thought of using the term enterochrome for this pigment, but there are several pigments to which that term would equally apply.

† They are stained also dark by logwood, and stained redder with eosin and safranin.

‡ PECK, “The Structure of the Lamellibranchiate Gill,” ‘Quart. Journ. Micro. Sci.’ 1876, vol. 17, p. 45.

first small, but in the ripe condition one large sphere can be seen almost filling the cell. This description applies word for word to the ferment cell in other Molluscan gastric glands. See fig. 12, Plate 3.

The gland ducts are lined with columnar epithelium possessing a striated margin, and are ciliated. Between them are granular cells, supposed by some to be "macroblasts." They certainly contrast strongly with the other lining cells of the acini in being strongly eosinophilous, and they give one the impression of having insinuated themselves between the epithelial cells (see fig. 1). I must, however, leave this much disputed point to be settled by others, as it does not bear directly on the present questions. We also find in the gastric gland of *Ostrea* very peculiar strands and islets of a tissue to which it is difficult to apply an appropriate name. In longitudinal, and indeed in transverse sections (figs. 2 and 3, Plate 1), we find cells applied to each other, forming a mosaic pattern, which cells *appear* to be of an epithelioid character.\* Where such strands approach the wall of the larger ducts, or that of the intestine, these cells seem to break away from each other, and, being freed from mutual pressure, they become rounded, and look like leucocytes or amœbocytes. Sections made at right angles to the course of these strands give one the impression that they are solid cylinders or rods, as shown in fig. 3, Plate 1, and here is another very interesting analogy between this gland and the vertebrate pancreas. The "intra-alveolar cell islets"† met with in the latter are apparently composed of the same kind of tissue as that forming the carotid and coccygeal gland, and that just described in the gastric gland of the oyster. I do not find this tissue in *Mytilus*, or in any other mollusc examined up to the present time. The occurrence of this tissue then emphasises more strongly the view now generally received that the invertebrate gastric gland is, in some of its functions, a pancreas.

We find no "lime cells" (Kalkzellen) in this gastric gland, and, as already stated, no ferment cells. The latter are absent also, according to FRENZEL,‡ in *Mytilus*, *Solecurtus*, *Solen*, *Lithodomus*, and *Cytherea*.

Abundance of leucocytes can be seen in these sections of the gastric gland of *Ostrea*, and it is doubtless partly through their agency that the coloured fatty and other matters are carried from the gut to be deposited in the pigment-containing cells of the connective tissue, and in the granular cells of the gland itself.

There is a very striking contrast in the appearance of the pigment found in the gastric gland of *Mytilus edulis* to that of *Ostrea*; thus in the former we find it not enclosed in definite cells, but it looks as if deposited at random, as it were, and scattered in the connective tissue, between the acini of the gland. It can also be

\* Polygonal from mutual pressure. I believe, to coin a new word, this is an amœbocytogenous tissue—a "lymphatic tissue," in fact.

† See SCHÄFER, in 'Quain's Anatomy,' vol. 3, Part IV., 10th ed., 1896, and *cf.* HARRIS and GOW, 'Journ. Physiol.,' London and Cambridge, vol. 15, No. 4 (Nov. 1893).

‡ *Loc. cit.*, p. 173.

easily seen in the lining epithelium of the intestine, just as in *Patella* (as will be referred to later on). And the impression one forms, after examining sections of the gastric gland of *Mytilus*, is that the pigment has been introduced directly by absorption from the intestine, in other words, it is not secretory.

Just as in *Ostrea*, so in *Mytilus*, none of the so-called ferment cells are met with, the only cell present being the granular one. The granules are of very small size and brownish, and their colour is due to entero-chlorophyll. In some of these cells FRENZEL found proteid masses of a feeble yellow colour, but this investigator had a great difficulty in distinguishing between granules and groups of a proteid nature, so he says, but this is not to be wondered at for obvious reasons.

Other lamellibranch mollusca were examined, but there is nothing of importance calling for remark here which has any bearing on the present question.

*The Gastric Gland of Gastropod Mollusca.*—In *Helix Pomatia*, BARFURTH,\* as already stated, described three kinds of epithelial cells in the so-called liver, namely, ferment cells, hepatic cells, and lime cells. These are easily recognisable in *Helix hortensis* and *Helix aspersa*, in *Limax* and *Arion*, all of which I have examined. Plate 2, figs. 4 to 8, show the general structure of, and the various kinds of gland cells in, the gastric gland of *Limax flavus*. The deeply-stained cells at the periphery of an acinus are supposed to be young cells and lime cells. If we use “the dark ground illumination,” for example, by putting a central stop in the substage condenser, the calcareous granules of these cells stand out white on the dark background, and they are thus, as well as by micro-chemical reactions, sharply differentiated from the other kinds of cells.

The sharp distinction between granular cells and ferment cells which BARFURTH and FRENZEL would draw, does not seem to me justified by a study of the gastric gland of *Helix* or *Limax*. For we find in a ferment cell, such as that shown in Plate 3, fig. 13, round granules, differing in no respect from similar granules in a granular cell, of which two are shown in the lower part of the same drawing.† It may also be stated here that the contents of the ferment cell and that of the granular cell when coloured greenish owes its colour to entero-chlorophyll.‡ The great distinction between a granular cell and a ferment cell would appear to be simply constituted by the presence of a vesicular-looking structure in the latter. Its contents may vary from small granules up to a large spherical mass of pigmented substance. In the granular cell, too, the contents may vary; thus sometimes small granules may be found, at other times the spheres may come to resemble closely those found in the ferment cells.

It is a mistake to suppose that the lime cells disappear from a fasting gland.

\* BARFURTH, *loc. cit.* Attention may also be called to the fine paper of LEVY on the Liver of *Helix pomatia*, dealing, however, with its chemical characters, ‘*Zeitsch. f. Biol.*,’ vol. 27, p. 398, &c.

† See also fig. 9, lower part.

‡ Perhaps mixed at times with other pigments.

They are still very abundant, apparently quite as abundant as in a gland in full metabolism during feeding.

Again, as shown in fig. 9, Plate 3, which represents the contents of the mid-gut in proximity to the gastric gland of *Helix hortensis*—which had been kept fasting for about eight weeks—we find not only the remains of ferment cells, and granular cells and lime cells, but also transitional forms which cannot be sharply classified either as ferment cells or granular cells. Such forms are shown in the lower part of that drawing. So that in Mollusca as in Crustacea we find transitional forms between the various kinds of cells.

It might be inferred from superficial examination, and from comparison with the fat-holding cells of the Crustacea, that these granules in the gland cells of the Mollusca are chemically similar, but this is not the case. These granules, and indeed the great oily-looking coloured masses in the ferment cells, are not composed of fat, but are of proteid nature. The following reactions were obtained with the gastric gland of *Limax flavus*.

(1) No starch, or cellulose, or glycogen could be detected in the gland anywhere. (2) The contents of the ferment cells were stained red by gentian violet, just as the coloured bodies in the vesicular connective tissue of *Ostrea* are coloured by that dye. (3) Eosin left the coloured contents of the ferment cells green. (4) Methyl green deepened the colour of these pigmented bodies. (5) Osmic acid, while staining, after prolonged action, the whole of the section brownish, produced no special blackening in the contents of the ferment cells. (6) Mucicarmine produced no staining of the latter; the periphery of the lobules, and here and there the connective tissue, seemed slightly stained. (7) The contents of the ferment cells were stained reddish by safranin and (8) greener by methyl green. (9) The contents of the ferment cells were disintegrated and dissolved by caustic soda and caustic potash, and to a less extent by ammonia.

In *Helix hortensis* the reactions obtained were practically identical. Thus (1) the same bodies were not stained by eosin or by hæmalum. (2) Methyl green, gentian violet, and other dyes produced the same effect as in *Limax*. (3) Osmic acid produced an identical effect, and so on.

In neither *Limax* nor *Helix* had acetic acid any effect on the contents of the ferment cells. In both, caustic soda disintegrated and dissolved them. Strong hydrochloric acid did not dissolve them. Acetic acid had apparently no effect, and even strong sulphuric acid had but little action at first. They became orange when treated with ammonia after the action of nitric acid.

It is interesting to note how like the chlorophyll bodies in plants these cell-inclusions are, as far as their chemical constitution is concerned, as both are of a proteid nature, impregnated with an oleaginous substance holding the pigment in solution, which latter is soluble in various fat-solvents, leaving the plasmic substance without colour.

I formerly stated\* that in *fasting* snails and slugs abundance of entero-chlorophyll can be found in the so-called liver. Anyone can see this for himself. Indeed there is almost as much pigment present both in the ferment and granular cells of a fasting gastric gland as in a feeding one. The only difference I can perceive is that in the fasting gland the outlines of the secreting cells are more difficult to make out than those in the feeding gland. Now with respect to fat I applied the new stain known as "Soudan III.," which stains fat a bright orange colour. While some of the contents of the ferment cells remained as before, in other cases a distinct coloration was produced, but the granular cells remained mostly unchanged. The gastric gland of a feeding snail seemed to contain quite as much, if not more, fat than a fasting one. The effect of mucicarmin on the gastric gland of the oyster and of *Limax* was referred to above. In the case of *Helix* I used thionin, but here again I could detect no appreciable amount of mucus. Certainly it is entirely absent from the contents of both ferment and granular cells. Curiously enough, however, some of the granular-looking contents of the lime cells of BARFURTH took on a violet-red coloration. However, FRENZEL found that these cells are stained at times by other reagents as well. The thionin produces a deep dark green in the pigmented contents of the ferment cells, but in using any stain we must remember that the resulting colour is due to the union of the natural colouring matter of these pigment bodies with that of the dye used. Hence also the difficulty of determining how much darkening is produced in them by the use of osmic acid.

I would now call attention to fig. 8, Plate 2, and figs. 9, 10, and 11, Plate 3. In fig. 9 we have the appearances seen when the contents of the intestine in the neighbourhood of the gastric gland of *Helix hortensis* is examined with a Zeiss apochromatic 1/12th and compensating ocular 8. Here there are present in the fluid poured into the intestine, *and which, therefore, must be in a great part excretory*, granular cells, ferment cells, and their separate contents, and granules from the calcareous or lime-holding cells, &c.

This fluid in fasting snails or slugs is of a reddish colour, and gives, when treated by such reducing fluids as ammonium sulphide or by Stokes's fluid, &c., the bands of hæmochromogen.† It is tempting at first to associate the presence of this entero-hæmatin with that of the ferment cells, which indeed in sections often have a reddish pigment in their interior, but such a hypothesis cannot be proved, because we cannot get the spectrum of hæmochromogen in the ferment cells, when sections are treated with reducing agents; and again, this pigment occurs in forms where no such ferment cells are found, for example, in *Patella*. In fig. 8, Plate 2, we see how all these different kinds of cells are shed into the lumen of an alveolus of the gastric gland of *Limax*, and one can hardly suppose that such cells can be of much further use in the metabolism of the animal. Again in *Aplysia* we find exactly the

\* 'Roy. Soc. Proc.,' *loc. cit.*

† See 'Phil. Trans.,' Part I., 1886, p. 240. This fluid is *acid* in reaction.

same cells in the gastric gland itself (when examined fresh in sea water or in *Aplysia's* own blood) as those found in the intestine, in proximity to the gastric gland, as shown in figs. 10 and 11, Plate 3. These observations—and many others of a similar kind might be cited—go to prove that the gastric gland performs an *excretory function*. A view which, indeed, BARFURTH held, but which FRENZEL strenuously endeavoured to set aside.\*

In the gastric gland of *Patella vulgata*, as stated before, no ferment cells are found, only FRENZEL's granular cells, but between these we find cells of a more or less triangular shape, whose protoplasm has a greater avidity for stains than the granular cells, these are placed peripherally in the acinus, and their apices do not generally reach as far as the lumen. Doubtless they are young cells, destined to replace the others, which latter are, when useless, shed into the lumen of the alveolus, from thence into the duct, and finally into the intestine.

Other points of great interest which are presented by the gastric gland of *Patella*, will be referred to later on.

I have made a number of observations on the gastric gland of *Aplysia punctata*, which is a very remarkable one in many points. It is an extremely difficult gland to fix, as already stated, and belongs to LEYDIG's spongy or cavernous type. Very numerous nerve ganglia can be seen in it, and its secreting cells are of three kinds, as FRENZEL points out, namely, granular cells, ferment cells (or club cells), and calcareous cells. The "ferment cells," however, are quite different in appearance from those called by that name in *Helix* or *Limax*. Their contents form the most striking object in sections of *Aplysia's* gastric gland. While their contents are generally of a brown colour, yet sometimes they appear purplish. This purplish colour seems associated with a similar colour in the integument of *Aplysia*, but has nothing to do with that peculiar colouring matter secreted by the dermal glands known as Aplysiopurpurin.

We also find orange hexagonal *crystals* very abundantly present in this gastric gland, which seem scattered over sections of it, but which I believe come mostly from the granular cells, and I think we find transitional forms between these crystals and certain granules, which appear to be of a proteid nature. While all the secreting cells are, *in situ*, more or less columnar in shape, yet when freed from mutual pressure, just as in the case of *Helix*, *Limax*, &c., they become quite spherical (see figs. 9, 10, and 11, Plate 3). The pigmented contents of the ferment cell is always, as a rule, more or less granular, and seems to be of a semi-fluid consistence. Some, however, seemed homogeneous in the quite fresh condition. The usual colour is greenish or brownish, or sometimes purplish, as already stated.

The following observations were made on the fresh glandular elements :—(1) Ether

\* FRENZEL's reason for doing so appears to have been mainly based on observations made by him on the intestinal contents of certain Mollusca; as he found the ferment cells lost their colour as they approached the end of the gut, and the fæces became white.



seemed to extract some colouring matter from the contents of the ferment cells, and after its action the orange crystals, owing perhaps to loss of colour, were not quite as perceptible. Some shrinking of the former also seemed to take place. (2) Alcohol also caused shrinking of the contents of the ferment cells. (3) Distilled water extracted some yellow colouring matter from the gland, and after its action many of the ferment cells contained less deeply coloured contents than before. This aqueous solution gave a band from  $\lambda$  510 to  $\lambda$  480, and possessed, like the fresh liver, an acid reaction. Curiously enough when this fluid was treated with ammonium sulphide a band appeared in the red from about  $\lambda$  640 to  $\lambda$  650, *i.e.*, very narrow. (4) When extracted with glycerin, the latter solvent became yellow or brown-yellow, afterwards becoming quite a dark brown; besides a feeble shading in the green, a band at the blue end of green was seen from about  $\lambda$  480 to  $\lambda$  510. The reaction of this glycerin extract was feebly acid. When treated with reducing agents no marked change in the spectrum took place. After the action of glycerin the contents of the ferment cells were seen to be shrunken and deformed, some being lighter, others darker in colour than before. (5) Caustic potash caused the complete destruction and disappearance of the coloured contents of the ferment cells, but did not seem to affect the granular cells. (6) Osmic acid blackened the former. (7) Acetic acid had little effect on them. (8) With hydrochloric acid—1 in 3—the contents of the ferment cells were unchanged. (9) Heating changed the colour of the latter, after strong hydrochloric acid had been added, to a greener colour. (10) Caustic soda, also ammonia, like caustic potash, caused their disappearance. (11) No colour change could be recognised in the gland by the action of iodine in potassic iodide, nor in its cell-inclusions: so that here, as in other gastric glands, no glycogen is present. (12) The contents of the ferment cells were stained red by gentian violet, dark red by safranin, dark green by methyl green, and were not stained by logwood. Finally, I think we may conclude that the ferment cell of *Aplysia* contains a body of a proteid nature, impregnated with an oleaginous substance, which latter holds entero-chlorophyll in solution; just as in the case of the Mollusca referred to above. The orange hexagonal crystals are also apparently of a proteid nature. I thought at first they were simply a crystallised lipochrome, but the application of various micro-chemical tests convinced me that they are of a proteid nature, and probably coloured by a lipochrome; but I may refer to FRENZEL's papers for further information on the various crystals met with by him in the gastric gland of other Mollusca and Crustacea.

There is no doubt that besides entero-chlorophyll which is abundantly present in the gastric gland of *Aplysia*, both in the granular and ferment cells, we have other pigments present also, one being soluble in glycerin and perhaps another in water.

I have made several experiments on the digestive ferments of the gastric gland

of *Aplysia*, and I arrived at exactly the same conclusion as recent observers who have worked at the same subject in other Mollusca, namely, that it is capable of digesting fibrin in acid and alkaline solutions, and of changing starch into sugar. We have, in fact, peptic, tryptic, and amylolytic ferments present.\*

I have not thought it necessary to describe in greater detail the other cell-inclusions found in the above or other gastric glands, because FRENZEL has, in his most recent papers, done this so thoroughly that he has left little to be described. He, however, draws, in my opinion, too hard and fast lines between the granular and ferment cells.

*The Gastric Gland of Decapod Crustacea.*—In those Decapod Crustacea which I have examined I have found much less entero-chlorophyll or other pigment than in the Mollusca. The gastric gland is of simpler construction, the cœcal tubes composing it are not as a rule bound to each other by connective tissue, but hang freely into the body-cavity. The kinds of cells found, and described by WEBER and FRENZEL, have been already referred to. These authors have also fully described the general structure of the gland.†

If we take the gastric gland of a prawn (*Palæmon*) and tease it up in sea water, we see numerous cœcal tubes filled with what looks like fatty matter, but also round large orange bodies, the ferment cells. Numerous free oil globules, soluble in ether and turpentine, are noticeable, these too are generally coloured yellow or greenish or orange. A reddish-yellow liquid can be squeezed out of the gland which, when examined with the spectroscope, gives some shading at the blue end of the green. In *Portunus depurator* the gland is yellow and in places greenish, and on teasing in sea water large yellow roundish clear looking vesicles, the ferment cells, are seen. Fat-holding cells are also abundant. The gland exudes a coloured juice which becomes (as in other Decapod Crustacea) darker on exposure to the air, and is here of a dirty green colour. In *Stenorhyncus* the tubes composing the gland are shorter than in the two former species, and contain the same kind of gland cells, the fat-holding cells filled with oil globules and the ferment cells with the large coloured (yellow) vesicles. In all these species it is easy to see that the contents of the ferment cell are impregnated by an oleaginous-like substance, which flows slowly out of the cells when compressed.

Osmic acid quickly darkens the contents of the fatty cells and more slowly that of the ferment cells. It is easy to see that the same pigment is present in either of these cells. It is not necessary to describe these gastric glands‡ more in detail, as they can all be referred to the type of the lobster whose gastric gland is figured in Plate 4, figs. 17 to 21. In fig. 17 we have an optical section of one of the cœcal tubes. In it, after fixation (by the method described) and staining with

\* The reaction of the gland itself is distinctly acid.

† The ferment cells seem to contain the bulk of the colouring matter.

‡ Of which permanent preparations were made by the methods described.

hæmalum followed by eosin, we find that some of the cells which look stellate have taken up the stain more than others; these are the younger cells (*y.c.*). A longitudinal section of a tube-wall, as shown in fig. 18, shows these cells in profile, some triangular only reaching up a short way between the other cells, others reaching to the lumen of the tube. Besides these we find the larger vesicular-looking ferment cell (*f.c.*) and the fat-holding cells (*d.g.c.*). The former seem to be more abundant in the middle of the tube represented in fig. 17.

Fig. 19, which represents these tubes cut transversely, shows three kinds of cells, and fig. 20 even better. Looking down on such cells as the young ones, and the fat-holding cells, cut transversely, we get the appearance shown in fig. 21.

Micro-chemical reactions:—

1. Osmic acid causes darkening of the fat cells first, afterwards of the ferment cell-contents.
2. When ether or benzol or other fat-solvent comes in contact with the fat-holding cells their contents are dissolved.
3. Only the usual yellow proteid reaction can be obtained when the gland is treated with iodine dissolved in potassic iodide: therefore, no glycogen is present.
4. No carbonate of lime is present in the gland cells.

In *Homarus*, as in the Mollusca, one can see shedding of the gland cells into the lumen of the gland tubes; not only are the fat-holding cells and the ferment cells shed, but also those which have been taken for young cells (*y.c.*). As already described by WEBER, and by FRENZEL, we find the portion of each cell next the lumen of the gastric gland tube finely striated and surmounted by a striated border from which cilia project into the lumen of the tube. This ciliation may be unperceived in balsam preparations, but can be easily seen in glycerin ones. Now in Mollusca ciliation is confined to the gland-ducts, the glandular epithelial cells—at least as far as my experience goes—being free from it. The pigment found in the fat-holding cell and that in the ferment cell appears to be identical, and as far as I have been able to ascertain is either a lipochrome or entero-chlorophyll or both.

*The functions of the Gastric Gland.*—It is not now necessary to state that the gland is in no way, except one, entitled to be called a liver, and that one is its capability of apparently forming, and certainly of storing, fat and pigment. One by one its pretensions to the “hepatic” function have been removed, and it finally has emerged from the discussion, as simply a pancreas. Now, it performs, in addition to its ferment-producing function (peptic, tryptic, and amylolytic, &c.), at least two other functions:—namely, it is as stated a *storer of fat and its associated pigment, or pigments*, and I am afraid in spite of FRENZEL’s opposition it must be credited with an *excretory* function. I have avoided reference to its lime-storing property, as BARFURTH, in spite of FRENZEL’s opposition, still clings to his own view, and I think BARFURTH was right. The questions now arise:—What is the nature of the

pigment found in the gland? Is it of a chlorophylloid nature? If so, how is it formed, how does it get into the gland? and so on.

In answer to the first question we must leave aside *qualitative* spectrum analysis as an authority, and appeal to spectro-photometry. The spectro-photometer is, I believe, the only instrument which allows one to prove the identity or non-identity of pigments with banded absorption spectra by means of numbers, and if we take the mean of several readings, which agree closely among themselves, there is not much room left for the "personal equation."

Yet, in deciding whether a pigment is chlorophylloid or not, we *must* appeal to the spectroscope. Considering that in spite of the great abundance of green plants on the earth no one has yet isolated pure chlorophyll, or determined its elementary composition, is it a matter of great wonder that pigments resembling it, but occurring in very small amounts, have not yet been prepared in a pure state, much less subjected to elementary analysis? Even plant chlorophyll cannot yet be obtained as a "thoroughly cleansed powder," much less in a crystalline state, although not a few observers have thought they had isolated pure chlorophyll in the crystalline condition. So that at present, since no combustions can be made of chlorophylloid pigments, what are falsely called their "chemical characters" relate simply to questions of solubility and reactions.

*Entero-chlorophyll and Plant Chlorophyll.*—In calling the pigment obtainable from the gastric gland of Invertebrates by the name entero-chlorophyll, I meant to express the idea forced upon me by an examination of most of the then known pigments of animals, and of many of those of vegetable origin, that this gastric gland pigment was nearer to vegetable chlorophyll than to any other. I was, however, quite aware of the fact that the spectrum of this substance was not quite identical with that of unchanged vegetable chlorophyll, as any one can see who refers to my papers. I now repeat that this entero-chlorophyll deserves its name, and my reasons for doing so are given below.

Before giving the results of spectro-photometric observations, I may here give the results of an examination of the entero-chlorophyll of *Aplysia punctata*, and of Chætopterin, to which latter Professor RAY LANKESTER has drawn renewed attention of late.\*

*Entero-chlorophyll of Aplysia.*—In the chart of spectra (p. 22), sp. 6 represents the absorption spectrum of an alcohol solution of the entero-chlorophyll of *Aplysia punctata*.

The measurements of the bands in a large chemical spectroscope were as follows :—

1st.	$\lambda$ 674 to $\lambda$ 641·5 . . . . .	centre at $\lambda$ 661.
2nd.	$\lambda$ 616 „ $\lambda$ 590·5 . . . . .	„ $\lambda$ 603·5.
3rd.	A shading $\lambda$ 571 to $\lambda$ 558 . . . . .	„ $\lambda$ 566.
4th.	$\lambda$ 547 to $\lambda$ 533 . . . . .	„ $\lambda$ 542.
5th.	$\lambda$ 518 „ $\lambda$ 497 . . . . .	„ $\lambda$ 507.

\* *Loc. cit.*

This fluid gives a fine red fluorescence, and is of a dull green or yellow-green colour. In the alcohol extract of the gastric gland of another *Aplysia* I noticed a band between the 1st and 2nd bands given above, from  $\lambda$  627 to  $\lambda$  619. In thinner layers there is also a band nearer the violet end. If now a drop or so of hydrochloric acid be added to this solution, very little change takes place in the colour, but we find the 5th band (from the red) almost gone, the 3rd band is no longer a mere shading but a distinct band, and the dominant band in red is slightly moved towards the violet. After a little time the fluid is of a bluer-green than before, but the red fluorescence is maintained. Compare sp. 6 and sp. 7 (p. 22).

These bands of acid entero-chlorophyll read as follows :—

1st.	$\lambda$ 670 to $\lambda$ 637 . . . . .	centre $\lambda$ 655 (or 653).
2nd.	$\lambda$ 614 „ $\lambda$ 591.5 . . . . .	„ $\lambda$ 602 (or 600).
3rd.	$\lambda$ 580 „ $\lambda$ 561.5 . . . . .	„ $\lambda$ 571.
4th.	$\lambda$ 545 „ $\lambda$ 524 . . . . .	„ $\lambda$ 535.
5th.	$\lambda$ 510 „ $\lambda$ 487 . . . . .	„ $\lambda$ 499 (?).

The action of caustic alkalies is not quite as satisfactory as in the case of Chætopterin, since turbidity is produced. The most remarkable appearance is the broadening of the 2nd band from the red.

The following are the readings of the bands in this spectrum :—

1st.	$\lambda$ 678 to $\lambda$ 637 . . . . .	centre $\lambda$ 661.
2nd.	$\lambda$ 627 „ $\lambda$ 592 . . . . .	„ $\lambda$ 616.
3rd.	An uncertain shading.	
4th.	$\lambda$ 550 to $\lambda$ 536.5 . . . . .	„ $\lambda$ 545.
5th.	From $\lambda$ 522 to an uncertain distance.	

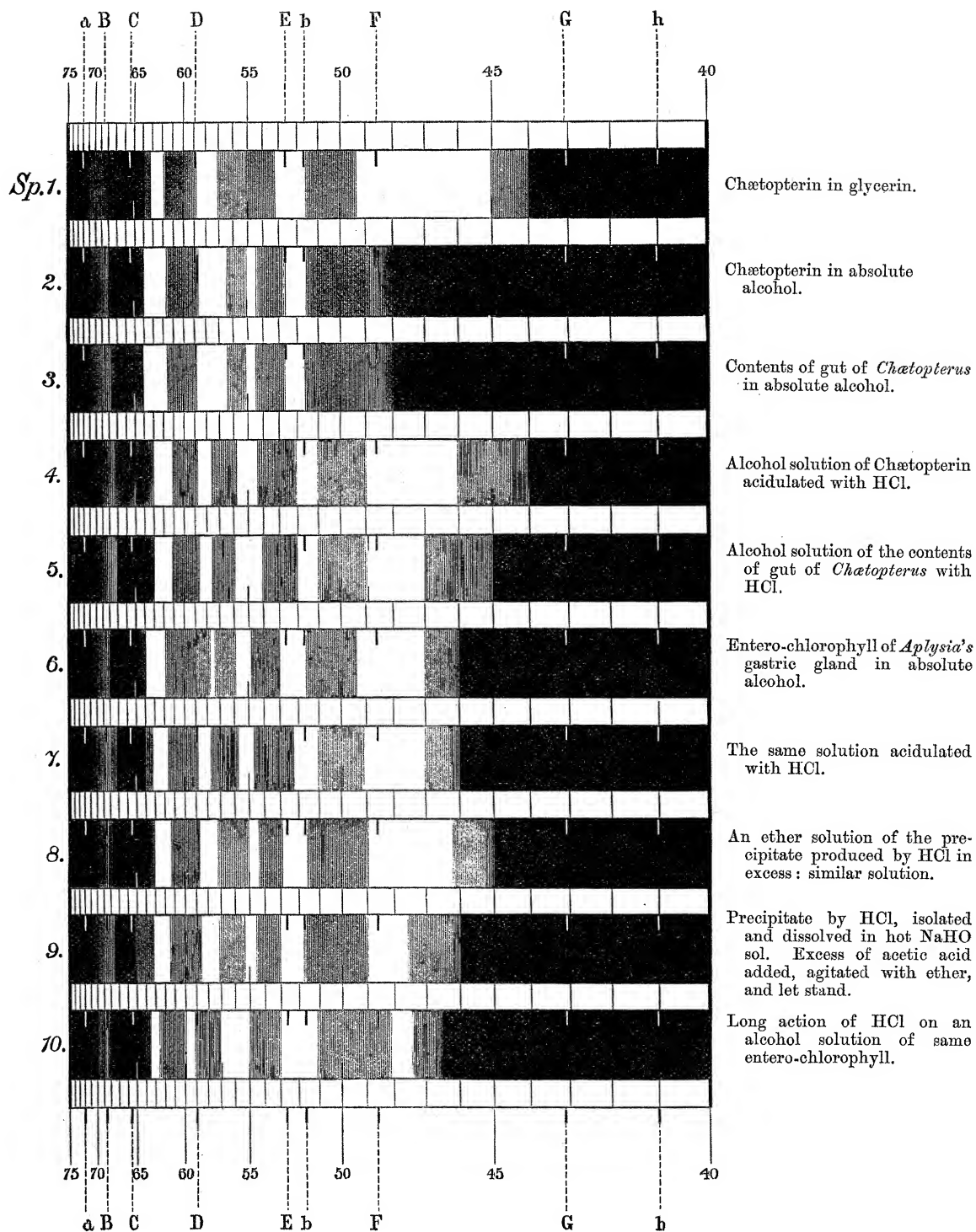
The above will suffice for enabling a comparison to be made with Chætopterin, but it may be stated here that whereas entero-chlorophyll is insoluble in glycerin, Chætopterin yields to glycerin a fine blue-green colour giving sp. 1.

*Does entero-chlorophyll yield the same decomposition-products as vegetable chlorophyll?*

Dr. SCHUNCK advises trying a few simple tests when in doubt as to the chlorophylloid nature of a pigment.\* Following his directions I added to a moderately strong, though I fear hardly strong enough, alcohol solution of *Aplysia's* entero-chlorophyll a few drops of hydrochloric acid and compared it with acid chlorophyll; the spectra were the same. (See sp. 7.) The same acid in larger quantity was then added, and the solution allowed to stand several days, a dark coloured precipitate formed, this was filtered off and dissolved in ether, when it gave sp. 8, which I take to be that of “phyllocyanin.” The rest of the deposit was now dissolved in “hot alkaline lye,” an excess of acetic acid added, and ether added to dissolve the flocc-

\* SCHUNCK, “The Chemistry of Chlorophyll,” ‘Annals of Botany,’ vol. 3, No. 9, 1899.

## CHART OF SPECTRA.



culent precipitate, which it easily did ; it was then allowed to stand for several days, and then gave sp. 9. Even if the spectra do not exactly correspond to those figured by Dr. SCHUNCK, and they do pretty closely, yet the reactions follow those of vegetable chlorophyll stage for stage. So far then there cannot be much doubt that the name entero-chlorophyll is correct. The long-continued action of hydrochloric acid produced sp. 10, which can be compared with the similar solution of vegetable chlorophyll, when I believe the last trace of doubt must disappear.

*Chætopterin and Entero-chlorophyll.*—Professor LANKESTER says it would be interesting to compare his Chætopterin with entero-chlorophyll ; this I have been enabled to do, not only by the usual spectroscopic method but by means of the spectrophotometer, and the result has fully realised expectation, as there is a very remarkable likeness, not only between the absorption curves but in the absorption spectra, and the change produced in these spectra by reagents. I was fortunate enough while working at the Plymouth Marine Laboratory last year to obtain some specimens of that strange worm, *Chætopterus*. The very close agreement between the spectra of Chætopterin and of entero-chlorophyll can best be appreciated by a study of the Chart of Spectra. *Cf., e.g.,* sp. 2 with sp. 6, and sp. 4 with sp. 7. The bands follow each other very closely, not only in position but in relative intensity of shading. I was, however, surprised to find that the contents of the gut, just at the place where the pigment is most abundant in its walls, gave to alcohol a greenish solution which showed a spectrum very like that of Chætopterin, thus an alcohol solution gave sp. 3. Comparing that with sp. 2 the result is very striking, but on adding an acid to this solution sp. 5 is obtained, which is again very like sp. 7. On examining the contents of the gut which yielded these results microscopically, I found diatoms and vegetable débris, but I could not detect the peculiar granules of Chætopterin which Professor LANKESTER found in the intestinal epithelium, and which he supposes are the forms in which Chætopterin occurs in the latter. Hence, then, there is a most remarkable agreement between Chætopterin and the food eaten by the worm, as far as the pigments are concerned. The impression left on the mind of an observer is that Chætopterin is taken up into the intestinal epithelium from the chlorophyll in the gut directly, *being, however, changed a little in the epithelial cells*. Of course the alternative is that the pigment found in the intestine is excreted into it, but the amount of pigment found in the gut is very much greater than can be accounted for by this hypothesis. Looking now at Professor LANKESTER's drawing (fig. 2, Plate 34 of his paper) one is at once struck by the fact that the pigment occurs *in those parts of the cells which are nearest to the lumen of the intestine*. I have myself prepared several preparations showing this, but I also find Chætopterin staining the protoplasm of the epithelial cells and dissolved in oil globules.\* That is, we find the pigment as we should expect to find it if absorbed from the intestine.

\* There are other appearances which suggest the presence of cells recalling to mind the ferment cells of the gastric gland ; these I have not yet studied.

As already stated, Chætopterin differs from entero-chlorophyll in being soluble in glycerin.

We may now compare the measurements of the bands of the various solutions :—

Entero-chlorophyll in absolute alcohol.	Chætopterin in absolute alcohol.	Contents of gut of Chætopterus in absolute alcohol.
Band.		
1st. $\lambda$ 674 to $\lambda$ 641·5 centre $\lambda$ 661	$\lambda$ 670 to $\lambda$ 639 centre $\lambda$ 657	$\lambda$ 670 to $\lambda$ 639 centre $\lambda$ 657
2nd. $\lambda$ 616 „ $\lambda$ 590·5 „ $\lambda$ 603·5	$\lambda$ 612 „ $\lambda$ 592 „ $\lambda$ 603·5	$\lambda$ 612 „ $\lambda$ 592 „ $\lambda$ 603·5
3rd. $\lambda$ 571 „ $\lambda$ 558 „ $\lambda$ 566	$\lambda$ 566 „ $\lambda$ 555 „ $\lambda$ 560	$\lambda$ 566 „ $\lambda$ 555 „ $\lambda$ 560
4th. $\lambda$ 547 „ $\lambda$ 533 „ $\lambda$ 542	$\lambda$ 545 „ $\lambda$ 531 „ $\lambda$ 538	$\lambda$ 545 „ $\lambda$ 531 „ $\lambda$ 538
5th. $\lambda$ 518 „ $\lambda$ 497 „ $\lambda$ 507	$\lambda$ 514 „ $\lambda$ 495 „ $\lambda$ 504	$\lambda$ 514 „ $\lambda$ 495 „ $\lambda$ 504

I cannot see the bands of a lipochrome in the case of Chætopterin, in which point\* it differs from entero-chlorophyll, which is generally accompanied by one. But that it is closely connected to the latter the above figures go to show. They, however, show more strongly the extraordinary agreement between the bands of an alcohol solution of Chætopterin and those of a similar solution of the contents of the intestine.

This agreement is even more strongly brought out by comparing the action of hydrochloric acid on all three solutions, as shown by the following figures :—

Acid Entero-chlorophyll.	Acid Chætopterin.	Acid contents of gut.
Band.		
1st. $\lambda$ 670 to $\lambda$ 637 centre $\lambda$ 655	$\lambda$ 665 to $\lambda$ 633 centre $\lambda$ 650·5	$\lambda$ 665 to $\lambda$ 633 centre $\lambda$ 650·5
2nd. $\lambda$ 614 „ $\lambda$ 591·5 „ $\lambda$ 602	$\lambda$ 609 „ $\lambda$ 587·5 „ $\lambda$ 598	$\lambda$ 609 „ $\lambda$ 587·5 „ $\lambda$ 598
3rd. $\lambda$ 580 „ $\lambda$ 561·5 „ $\lambda$ 571	$\lambda$ 577 „ $\lambda$ 556·5 „ $\lambda$ 567	$\lambda$ 577 „ $\lambda$ 556·5 „ $\lambda$ 567
4th. $\lambda$ 545 „ $\lambda$ 524 „ $\lambda$ 535	$\lambda$ 542 „ $\lambda$ 522 „ $\lambda$ 533	$\lambda$ 542 „ $\lambda$ 522 „ $\lambda$ 533
5th. $\lambda$ 510 „ $\lambda$ 487 „ $\lambda$ 499 (?)	$\lambda$ 512 „ $\lambda$ 492 „ $\lambda$ 501	$\lambda$ 512 „ $\lambda$ 492 „ $\lambda$ 501

These figures show the absolute identity of the spectra of Chætopterin and of the intestinal chlorophyll of that worm, and the close similarity between entero-chlorophyll and Chætopterin. I do not think it necessary to give any more results of a *qualitative* kind, but I shall now show how, by *quantitative* methods, we arrive at very important conclusions.

*Spectrophotometric Measurements of Entero-chlorophyll, Plant Chlorophyll, and Chætopterin.*—The spectrophotometer used in my observations is a modification of that introduced by VIERORDT.† The modification consists of improvements introduced,

\* As well as being soluble in glycerin.

† ‘Die Anwendung des Spectralapparates zur Photometrie der Absorptions-spectren und zur quantitativen Chemischen Analyse,’ Tübingen, 1873. Also ‘Die quantitative Spectral-analyse in ihrer Anwendung auf Physiologie, Physik, Chemie und Technologie,’ Tübingen, 1876.



I believe, by the brothers KRÜSS.\* Thus the jaws of the double slit open symmetrically to the optic axis, and the glass cell is provided with a glass wedge 10 millims. thick. Various other improvements have been made for me by Mr. Adam Hilger, of London, to which I need not further refer, except to thank him for the excellent way in which he performed a difficult task. In this instrument the size of the aperture (of slit) is reduced or increased 0.00254 millim. when the graduated drumhead of screw is turned through one division. When the drumhead stands at 100, at which number we start to make the calculation, the aperture of slit is 0.254 millim. I have given the percentages of the *unabsorbed light*, not the co-efficients of extinction, as this is more convenient for the construction of curves, and is the method adopted by Professor ENGELMANN.†

The first point to be decided is this:—What is the difference shown by the spectrophotometer between plant chlorophyll and entero-chlorophyll? The reply is a considerable difference in the respective curves, but, and here comes the most interesting fact, *if we change plant chlorophyll into the modified pigment, or what is practically the same thing, into the slightly acid modification, by adding a little acetic acid and allowing it to stand some hours (about twenty-four), the curve follows closely, in its maxima and in its minima, expressing of course the maxima and minima of absorption of light by the solution, the curve of entero-chlorophyll and of Chætopterin.* It is necessary to give the acid time to act on the plant chlorophyll, as the curve is at first not quite the same as that obtained when the action has gone on for some hours. I used an absolute alcohol solution of *Nasturtium* leaves prepared in the cold. I need not give the numbers expressing the percentage of unabsorbed light in the case of an alcohol solution of plant chlorophyll, because we cannot take exactly the same regions of the spectrum for comparison with either entero-chlorophyll or Chætopterin. I have chosen Professor ENGELMANN'S spectrum regions for the latter, and the following numbers give the percentages of unabsorbed light. Each measurement was repeated at least five times, or more frequently, if necessary, and of course the mean taken.

\* 'Kolorimetrie und quantitative Spektralanalyse, &c.,' Hamburg and Leipzig, 1891.

† See Professor LANKESTER'S paper, *loc. cit.*, p. 461, &c.

TABLE showing Percentages of Unabsorbed Light in Solutions mentioned.

Modified chlorophyll of plants.		Entero-chlorophyll of <i>Aplysia</i> .		Chætopterin.	
	Per cent.		Per cent.		Per cent.
$\lambda$ 700	80.0	$\lambda$ 700	68.2	$\lambda$ 700	75.1
$\lambda$ 680	21.8	$\lambda$ 680	21.8	$\lambda$ 680	38.1
$\lambda$ 670	8.5	$\lambda$ 670	7.4	$\lambda$ 670	16.5
$\lambda$ 655	7.8	$\lambda$ 655	7.2	$\lambda$ 655	7.2
$\lambda$ 640	19.0	$\lambda$ 640	21.8	$\lambda$ 640	23.7
$\lambda$ 625	30.0	$\lambda$ 625	29.6	$\lambda$ 625	35.0
$\lambda$ 600	29.9	$\lambda$ 600	24.8	$\lambda$ 600	20.4
$\lambda$ 580	41.0	$\lambda$ 580	32.8	$\lambda$ 580	42.5
$\lambda$ 570	47.0	$\lambda$ 570	32.8	$\lambda$ 570	47.3
$\lambda$ 560	42.0	$\lambda$ 560	28.6	$\lambda$ 560	42.0
$\lambda$ 535	29.4	$\lambda$ 535	18.2	$\lambda$ 535	17.5
$\lambda$ 520	36.4	$\lambda$ 520	27.8	$\lambda$ 520	29.0
$\lambda$ 507	23.0	$\lambda$ 500	15.8	$\lambda$ 500	14.8
$\lambda$ 480	9.0	$\lambda$ 480	12.8	$\lambda$ 480	27.0
$\lambda$ 460	2.5	$\lambda$ 460	10.0	$\lambda$ 460	30.3
$\lambda$ 440	—	$\lambda$ 440	5.0	$\lambda$ 440	14.3
$\lambda$ 420	—	$\lambda$ 420	—	$\lambda$ 420	4.0

Spectrophotometric Curves of Chlorophyll, Entero-chlorophyll, and Chætopterin. Absolute alcohol solutions.

Abscissæ = wave-lengths of light in millionths of a millimeter.

Ordinates = percentages of unabsorbed light.

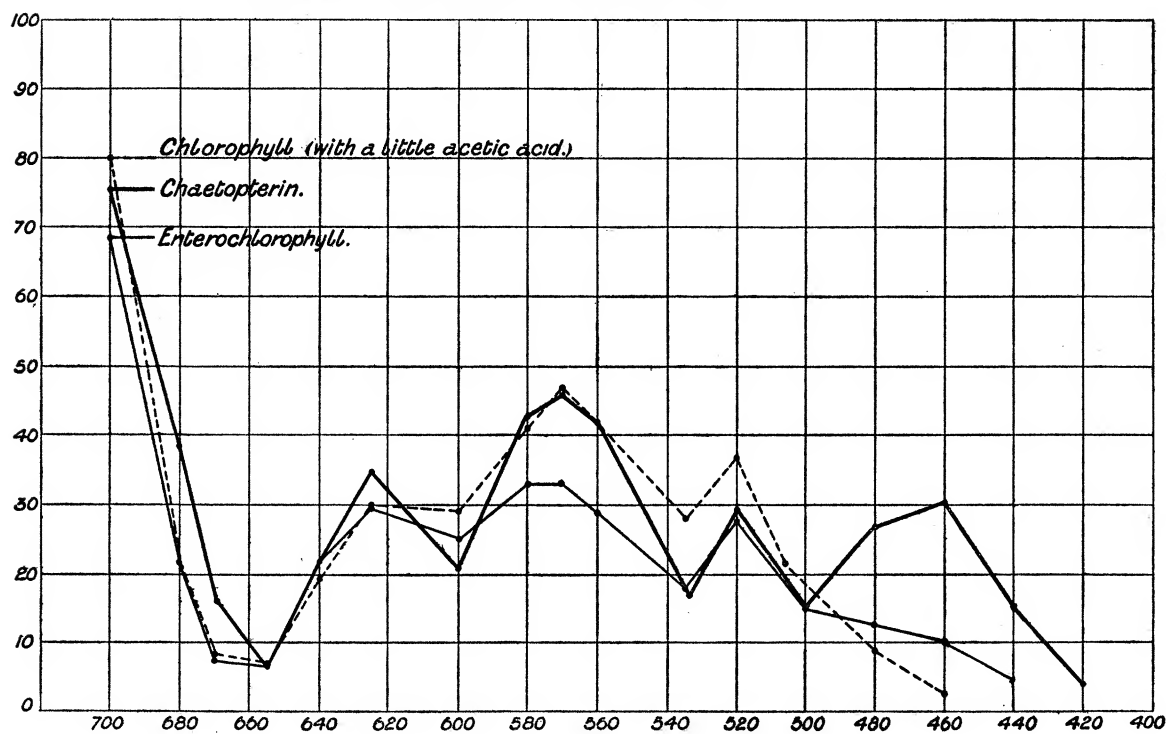


Chart of Curves I.

An inspection of "Chart of Curves I." will, however, convey to the eye a more striking representation of the connection between these pigments, and there we notice that just as Chætopterin is of a bluer tinge than entero-chlorophyll, so accordingly the latter absorbs more light at the blue end of the spectrum, while the former lets more through between  $\lambda$  500 and  $\lambda$  460. And this corresponds with what I have already stated that entero-chlorophyll is generally mixed with a lipochrome or lipochromes, whereas Chætopterin is free from such.

We may now compare by means of this method a solution of plant chlorophyll, treated with hydrochloric acid in absolute alcohol solution, with a similar solution of entero-chlorophyll. The result is as follows :—

TABLE showing Percentages of Unabsorbed Light in Solutions mentioned.

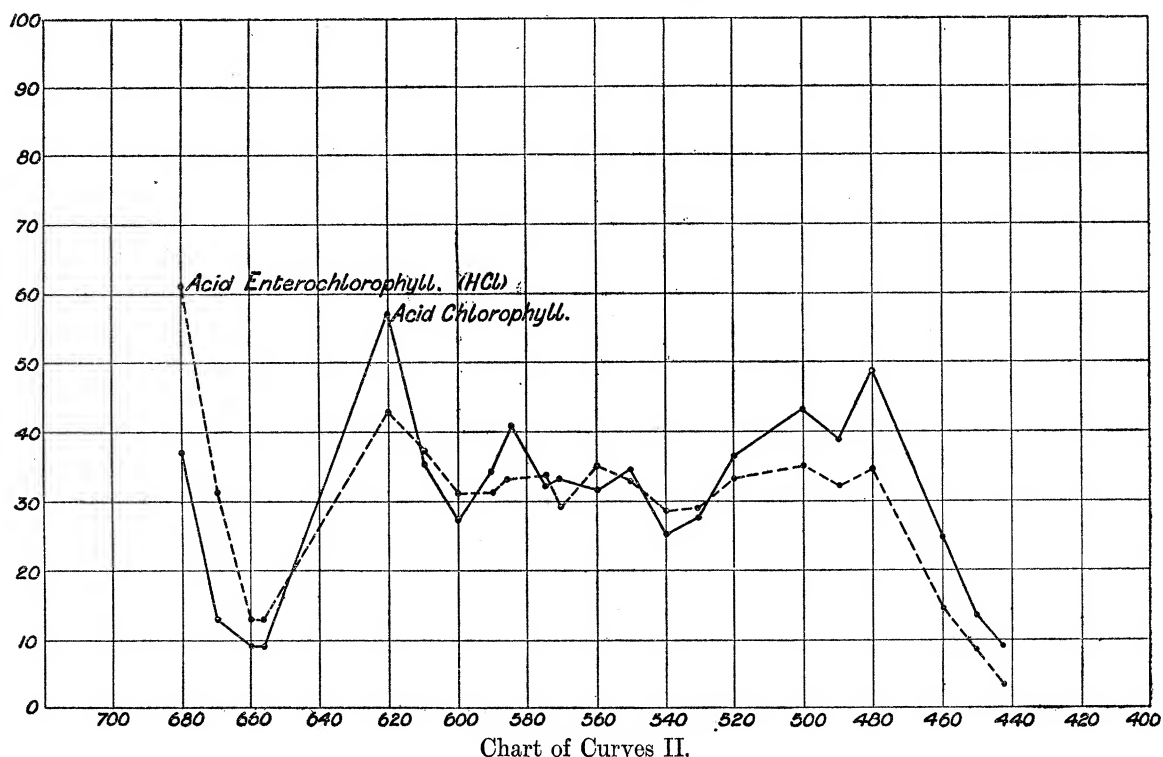
Plant chlorophyll + HCl.		Entero-chlorophyll + HCl.	
	Per cent.		Per cent.
$\lambda$ 680	36.6	$\lambda$ 680	60.7
$\lambda$ 670	13.0	$\lambda$ 670	31.3
$\lambda$ 660	9.0	$\lambda$ 660	13.0
$\lambda$ 656	9.0	$\lambda$ 656	12.5
$\lambda$ 620	57.3	$\lambda$ 620	42.5
$\lambda$ 610	35.2	$\lambda$ 610	37.8
$\lambda$ 600	27.0	$\lambda$ 600	31.0
$\lambda$ 590	34.3	$\lambda$ 590	31.3
$\lambda$ 585	41.0	$\lambda$ 585	32.3
$\lambda$ 575	32.5	$\lambda$ 575	33.6
$\lambda$ 570	33.0	$\lambda$ 570	29.0
$\lambda$ 560	31.8	$\lambda$ 560	35.0
$\lambda$ 550	34.3	$\lambda$ 550	33.3
$\lambda$ 540	25.3	$\lambda$ 540	28.5
$\lambda$ 530	27.3	$\lambda$ 530	28.6
$\lambda$ 520	36.5	$\lambda$ 520	33.5
$\lambda$ 500	43.0	$\lambda$ 500	34.8
$\lambda$ 490	38.5	$\lambda$ 490	32.0
$\lambda$ 480	49.3	$\lambda$ 480	34.0
$\lambda$ 460	24.0	$\lambda$ 460	14.0
$\lambda$ 450	13.8	$\lambda$ 450	8.8
$\lambda$ 442	9.0	$\lambda$ 442	3.0

An inspection of "Chart of Curves II." will show to anyone accustomed to this kind of work the close resemblance between these curves ; they follow each other up and down very closely, and the pigments must certainly be very closely connected chemically with each other. I may state further that I had not a sufficiently strong solution of entero-chlorophyll to work with as compared to the solution of *Nasturtium* chlorophyll. Otherwise, I am sure the results would have been more striking. Still, the results are good enough for the purpose, and show, as spectrophotometry can show, that entero-chlorophyll is at least a modified chlorophyll.

Spectrophotometric Curves of Acid Chlorophyll and Acid Entero-chlorophyll. Absolute alcohol solutions.

Abscissæ = wave lengths of light in millionths of a millimeter.

Ordinates = percentages of unabsorbed light.



*Origin of Entero-chlorophyll in the Gastric Gland.*—The obvious conclusion, then, from these observations made by this beautiful method of spectrophotometry, is that entero-chlorophyll is a chlorophyll,\* which has been modified by some external agency acting upon it, such as a weak acid or a ferment acting in a feebly acid medium. And I fear we must look to the intestine of the Mollusc or the Crustacean for its source. I am reluctantly compelled to adopt this view, as I had fondly hoped that it is built up in the gland itself from some other substance. However, now that the evidence points in this direction, let us see if we have other proof from an independent source. An inspection of Plate 4, figs. 14, 15, and 16, teaches one a good deal. Fig. 14 is a villus, or rather pseudo-villus, from the glandular stomach ("manyplies") of *Patella vulgata*, the cavity of which is filled with leucocytes (amœbocytes), some of which are seen insinuating themselves between the cells of the intestinal epithelium. In fig. 15, from another part of the intestine of *Patella*, we actually see the entero-chlorophyll within the columnar epithelial cells†; and, in fig. 16, the same thing is

\* It remains yet to compare the spectrophotometric measurements of solutions of the kind of chlorophyll eaten by each species of Mollusc, &c., with the pigments found in its gastric gland. I hope to undertake this investigation shortly.

† *Mytilus* presents the same appearance, and perhaps other Molluscs if examined.

seen, only better, as the magnification is much greater. Further, what I take to be eosinophilous leucocytes are seen at the base of attachment of these epithelial cells, forcing their way up between the cells. Here, then, we have an explanation of how entero-chlorophyll gets into the gastric gland. It is probably taken up from the intestine in small granules of a fatty nature, as can be easily proved, being held in solution in this fatty material. These fatty, coloured granules are then probably taken up by the leucocytes (or amœbocytes) and carried away to be deposited in the gastric gland and elsewhere. Of course the question arises: Is there a more direct absorption of colouring matter from the intestine into the gland? To this question I cannot reply. Some experiments made by C. DE SAINT HILAIRE\* bear, somewhat indirectly it must be said, on this point. Thus, he found that when a crayfish was injected *per anum* with solutions of certain colouring matters, some of these had stained the tubes of the gastric gland. However, we cannot say that these colouring matters are not carried by means of the blood to the gland. When he injected similar colouring matters into the blood directly, he found that they were excreted by means of the gastric gland, and after the absorption of the colouring matter, as SAINT HILAIRE thinks, by the blind ends of the glandular tubes, *the ferment cells of FRENZEL became coloured*; some time afterwards these coloured cells were found in the gastric juice and in the intestine. The absorbent action on the part of the secreting cells is a function of the living intact gland cell, as SAINT HILAIRE shows. He has also come to the conclusion that the gastric gland of the crayfish has an excretory and absorbent function, and he believes that digested food passes into its tubes.

CUENOT† made feeding experiments on crayfish by means of food coloured by various aniline dyes, which were afterwards found to colour the cœcal tubes of the gastric gland, and finally occurred in the fæces. He believes the gastric gland is an accumulator of reserve products, absorbs the soluble products of digestion, and is a regulator of the amount of water contained in the blood.

Both the observers named, of course, accept the pancreatic function of the gland.‡

We may take it then, I presume, as proved that colouring matters are taken up from the intestine and accumulate in the gland. But in the case of entero-chlorophyll we meet with a difficulty: the case is not as easily explained as it seems at first. All those who have worked at the histology of the gland agree in thinking that the pigment is a *secretion* on the part of the cells lining the cœcal tubes, alveoli, or acini, &c. And in the early stages of its formation or deposition as grains, or granules, &c., it seems almost colourless, then yellow, and finally dark green or brown,

\* 'Bull. Ac. Roy. Belgique,' vol. 62 (1892), pp. 506-16.

† "Études physiologiques sur les Crustacés Décapodes," 'Arch. Biol.,' vol. 13 (1894), p. 245, &c.; also 'Comptes Rendus,' vol. 116 (1893), p. 1257, &c.

‡ See also 'Journ. Roy. Micros. Soc.,' 1893, p. 427, &c., for a paper by H. M. BERNARD on digestion in spiders.

while the round deposits get larger and larger, and so on. The same remark applies to those curious deposits of pigment in LEYDIG'S "vesicular" connective tissue in the oyster, as referred to above.

If we were to assume that the entero-chlorophyll is present as a chromogen, perhaps formed by the action of a ferment on the gut-chlorophyll, we could meet the difficulties of the case, but we have at present no facts to support any such assumption.

However, I am sure we are safe in assuming that this pigment arrives at the gastric gland through the instrumentality of fatty matters, and it is deposited in the gland with other reserve products, and is finally, in part at least, excreted with the fæces. Whether or not it gives rise to the formation of other pigments, we cannot at present say.

*The Analogy between Entero-chlorophyll and some other Chlorophylloid Pigments met with in Animals.*

The lipochromes and entero-chlorophyll seem, according to all who have investigated the subject, to be closely connected. This view, no doubt, has primarily arisen from the intimate association between these pigments, and from the fact that they are soluble in the same fat solvents.

The extraordinary facility with which the lipochromes are transported from one part of a plant to another, seems to characterise the lipochromes in their transit through the animal body; of this, numerous instances have been furnished within recent times.\* The transit of a lipochrome or of a chlorophylloid pigment from the gut, elsewhere, is not to be explained by diffusion, it depends upon solubility in fat-dissolving media, and stands upon quite a different basis to the diffusion of such a pigment or pigmented proteid as hæmoglobin. Again, we have, as a rule, to look at the leucocytes or amœbocytes as the carriers of fat and fat-pigments, whereas these cells show no special preference for carrying hæmoglobin. We may take it for granted that wherever lipochromes can be carried by the blood, there a chlorophyll derivative can be carried also. This accounts for the presence of chlorophyll derivatives in such places as the (decalcified) shell of the shore-crab (*Carcinus mænas*), and in the integument of *Aplysia punctata*, where I have recently found them. No doubt if sought for systematically such instances would become more numerous.

Two other known instances may be mentioned, viz., the occurrence of a chlorophyll derivative in the elytræ of Cantharides beetles, as shown by POCKLINGTON,† and confirmed by me,‡ and that of POULTON'S "metachlorophyll" in the blood of certain lepidopterous larvæ. The "melanosis" which occurs when the blood of the latter is exposed to the air is not due, however, to the chlorophylloid constituent, but to

\* And of transit from plant to animal.

† 'Pharm. Jl. Trans.,' vol. 3, pp. 681 and 949.

‡ 'Brit. Assoc. Rep.,' 1883; and 'Proc. Birmingham Phil. Soc.,' vol. 5.

admixture with a "uranidine," as shown by KRUKENBERG,\* and more recently by CUENOT,† in the case of other insects' blood.

Other instances of the indestructibility of *modified* chlorophyll might be brought forward, but for the present the above will suffice to prove those points for which I have endeavoured to contend, and which I hope I have proved in this paper.

In conclusion, I beg to thank the Government Grant Committee of the Royal Society for a grant towards the purchase of an Apochromatic 1/12 objective, &c.; and I wish also to thank Mr. Allen, the Director of the Marine Biological Laboratory at Plymouth, for his kindness to me while working in the Laboratory there.

### *Supplementary Note.*

Just as I had finished this paper, I received a reprint of Miss NEWBIGIN's paper "On Certain Green (Chlorophylloid) Pigments in Invertebrates."‡ While I agree with the writer as to the action of reagents on, and certain other chemical characters of, the pigments, which have been carefully studied, I disagree as to the interpretation of results. Thus Miss NEWBIGIN believes that entero-chlorophyll is *excreted* by the intestinal epithelium, while I believe it is *absorbed* there. I have shown *where* it is excreted, namely, in the gastric gland itself: the pigment-carrying cells being shed into the lumen of the alveoli, or tubes, &c., from thence into the ducts, and thence into the intestine.

The really important point is this: Is entero-chlorophyll derived from food chlorophyll? And is it a decomposition product of chlorophyll? I leave this question to answer itself in the foregoing pages. It is interesting that an independent observer should have chosen *Patella's* intestine and figured it, as I have done; these observations having been made independently of each other.

### EXPLANATION OF THE PLATES.

(For Methods of Preparation see Text.)

### PLATE 1.

Fig. 1. Section through gastric gland of *Ostrea edulis*, showing in one drawing all the principal parts of the gland; *l.d.*, a large duct showing ciliation, striated border, lining of columnar epithelial cells with eosinophilous granular cells between them: the so-called macroblasts; *s.d.*, small duct; *l.s.d.*, longi-

\* 'Vergl. Physiol. Vorträge,' vol. 3 (1886).

† "Études sur le sang," &c., 'Arch. de Zool. Exp. et Gen.,' 2me série, vol. 9. For POULTON's papers, see 'Roy. Soc. Proc.,' vol. 38, 1885, and vol. 54, 1893.

‡ 'Quart. Jl. Micro. Sci.,' vol. 41, p. 391, &c.

tudinal section of a large duct; *c.t.*, connective tissue layer or tunica propria; *leuc.*, leucocytes; *v.c.t.*, "vesicular" connective tissue; *pigt. c.*, pigment cells. *b.v.*, blood vessel; *alv.*, section of an alveolus or tube; *macr.*, supposed "macroblasts"; *e.p.c.*, the peculiar epithelioid-celled tissue, seen in fig. 2.  $\times 260$ .

Fig. 2. Longitudinal section of a strand or cylinder of the epithelioid-celled tissue.  $\times 700$ .

Fig. 3. Section of such a strand showing its solid or rod-like character. It is seen surrounded by the vesicular connective tissue of LEYDIG. Two of the cells of the latter containing pigment bodies.  $\times 400$ .

(All stained with hæmatoxylin and eosin.)

### PLATE 2.

Fig. 4. Somewhat oblique section through an alveolus of the gastric gland of *Limax*; *y.c.*, young cells; *d.g.c.*, digestive gland cells: equivalent to the hepatic cells of MAX WEBER, or granular cells of FRENZEL; *f.c.*, ferment cells; *c.t.*, connective tissue; *c.c.*, calcareous cells with deeply staining protoplasm.  $\times 250$ .

Fig. 5. General structure of gland with a low power.  $\times 70$ . Lettering as before.

Fig. 6. The glandular epithelium under a higher power, showing clear vesicle and coloured sphere in the ferment cells, *f.c.*, and the granular cells, *d.g.c.*  $\times 450$ .

Fig. 7. The beginning of a gland duct and its continuation into an alveolus. Lettering as before. The ciliation of the duct is not shown, being hidden by the balsam.  $\times 110$ .

Fig. 8. This illustrates how all kinds of cells, viz., ferment cells, *f.c.*, granular cells, *d.g.c.*, and calcareous or lime-containing cells *c.c.* are shed off into the lumen of an alveolus and pass into the intestine. A celloidin preparation.  $\times 260$ .

(All stained with hæmatoxylin and eosin, and all from *Limax*.)

### PLATE 3.

Fig. 9. Contents of the mid-gut into which the ducts of the gastric gland open in *Helix hortensis*. In their natural colour. The snail had fasted more than six weeks. All three kinds of cells are represented either as such or by their contents; *d.g.c.*, digestive gland cell or granular cell; *s.f.c.*, spherical or other shaped pigmented mass from the ferment cells. Some very slightly coloured bodies from same source are also present. At the bottom of the figure are two cells, which I take to be transition forms between the granular and ferment cells. *l.gr.*, lime granules from the calcareous cells, which FRENZEL says he never could find in the intestine. Other granules



are also present. *y.c.*, a young cell.  $\times 1000$ . (ZEISS Apoch. 1/12, comp. ocular 8.)

Fig. 10. From the fresh gastric gland of *Aplysia punctata* teased out in sea water. *d.g.c.*, granular cell; *f.c.*, ferment cell contents; *cry.*, crystals.  $\times 1000$ . (ZEISS Apoch. 1/12, comp. ocular 8.)

Fig. 11. Also from *Aplysia punctata*. Contents of intestine close to gastric gland. *d.g.c.*, granular cell; *y.f.c.*, probably a ferment cell in an early stage; *p.m.*, the pigmented contents from a similar cell; *f.c.*, contents of an adult ferment cell; *cry.*, crystal.  $\times 1000$ . (ZEISS Apoch. 1/12, comp. ocular 8.)

Fig. 12. The pigmented spheres found in the connective tissue (LEYDIG'S vesicular tissue) of *Ostrea edulis*, to be compared with the similar contents of the ferment cells of the gastric gland of other Mollusca. The two upper cells have a resemblance to transition forms, sometimes met with in the glandular epithelium of the gastric gland of other Mollusca.  $\times 1000$ . (ZEISS Apoch. 1/12, comp. ocular 8.)

Fig. 13. A ferment cell, *f.c.*, with a large vesicle containing small greenish-yellow granules quite similar to some of those in the granular cells, is seen above, and two granular cells below. The protoplasm of *f.c.* is pushed against the periphery of the cell and towards its attached border (to the right). The nucleus occupies the latter part. A young spherical cell with three coloured spheres in its interior occupies the basal protoplasm of the cell. Note the granular character of this protoplasm.  $\times 1000$ . (ZEISS Apoch. 1/12, comp. ocular 8.)

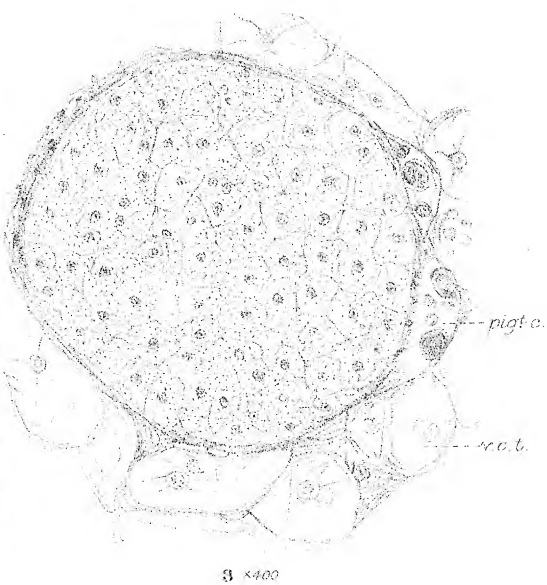
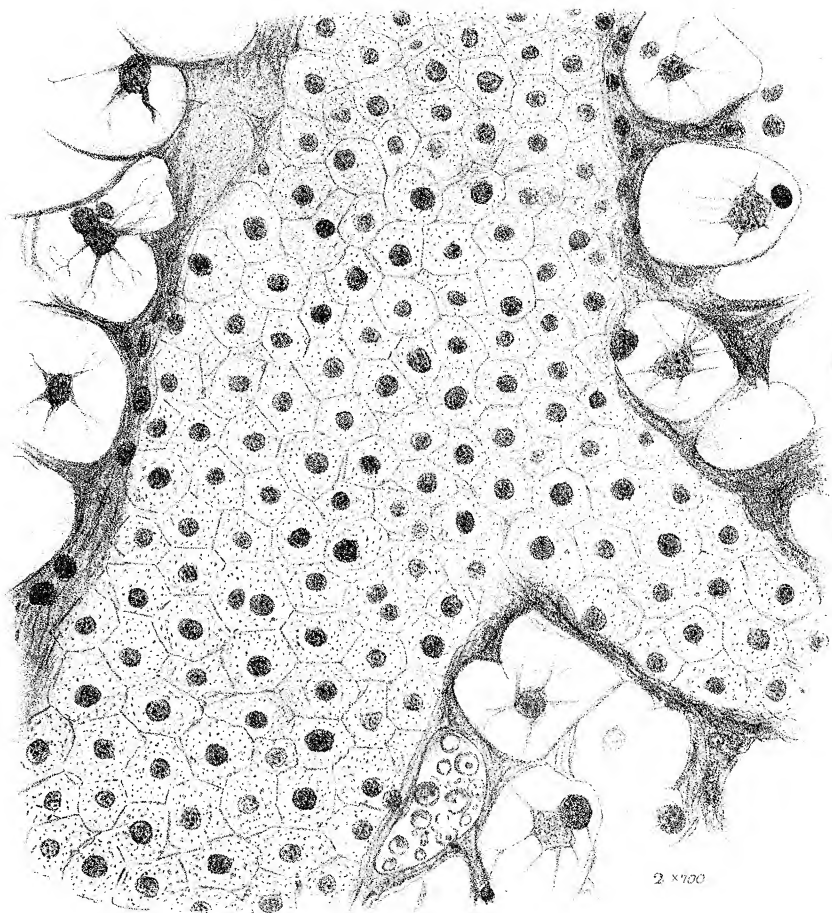
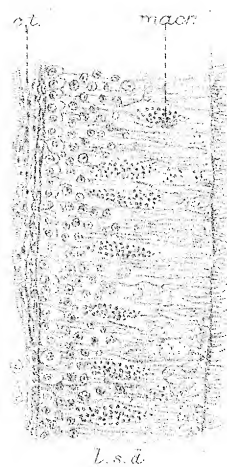
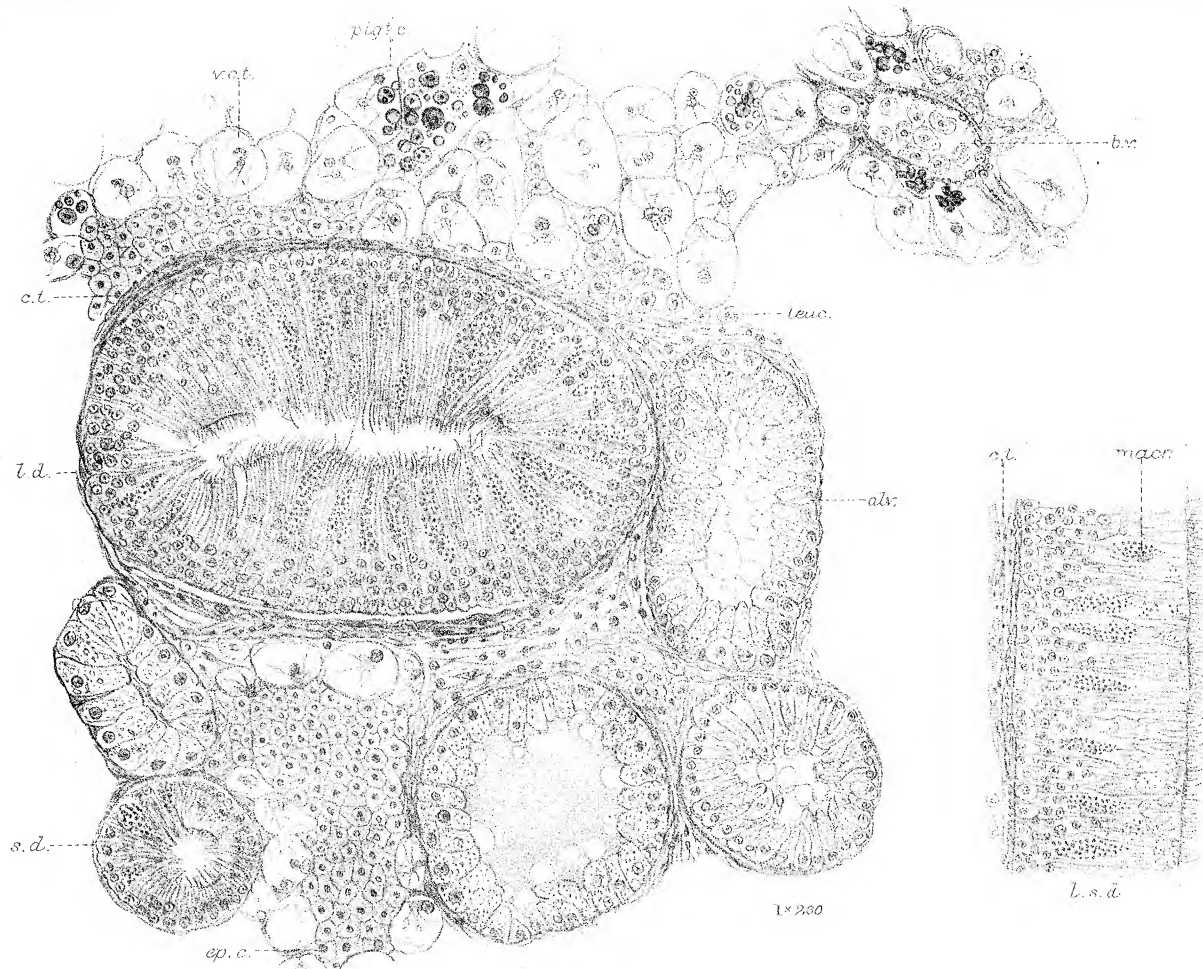
#### PLATE 4.

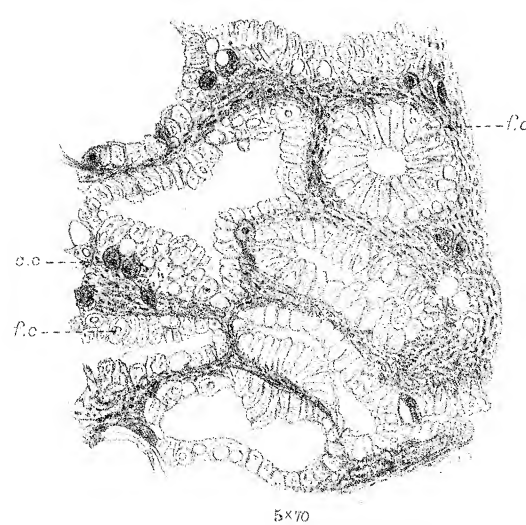
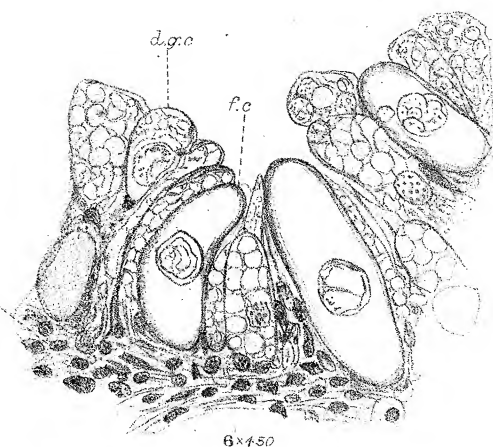
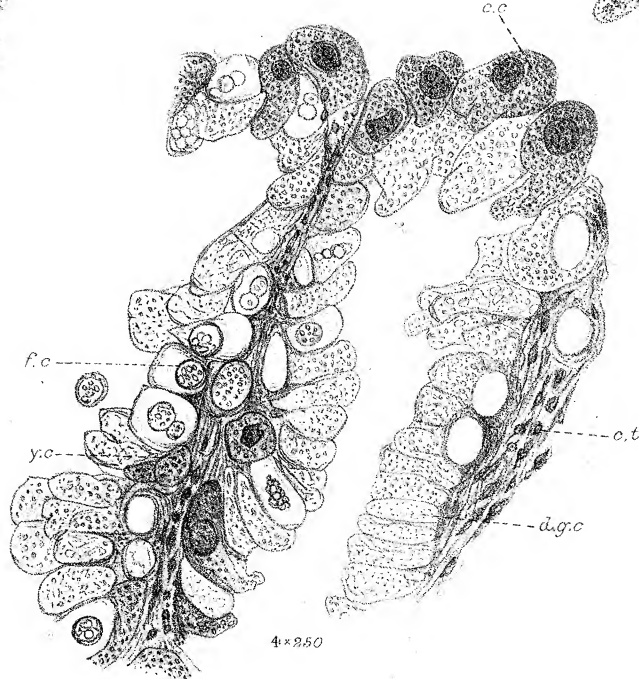
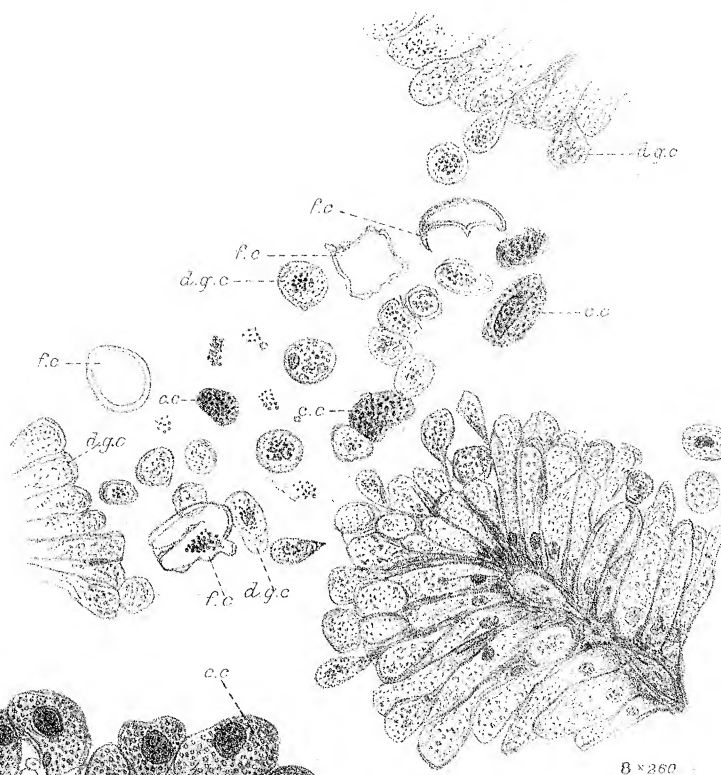
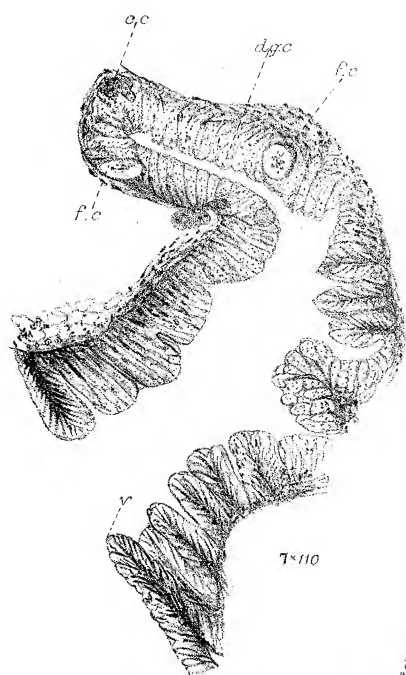
Fig. 14. A villus or pseudo-villus of the glandular stomach ("manyplies") of *Patella vulgata*: showing leucocytes filling the cavity of the villus, and some apparently pushing their way up between the columnar epithelial cells.  $\times 1000$ . (ZEISS Apoch. 1/12, comp. ocular 8.)

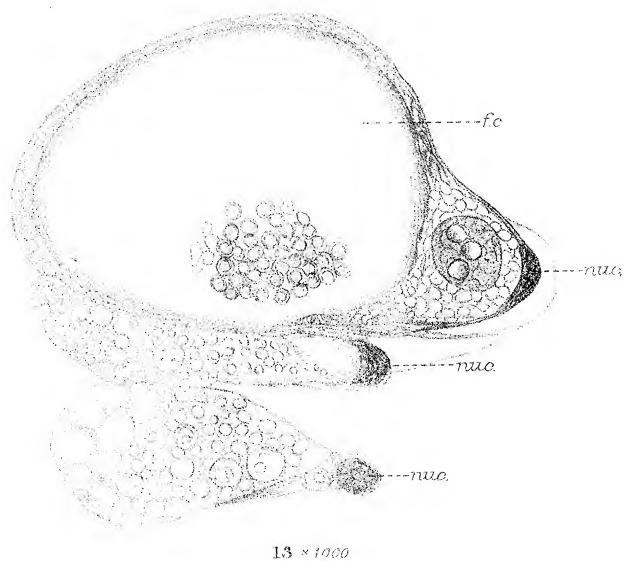
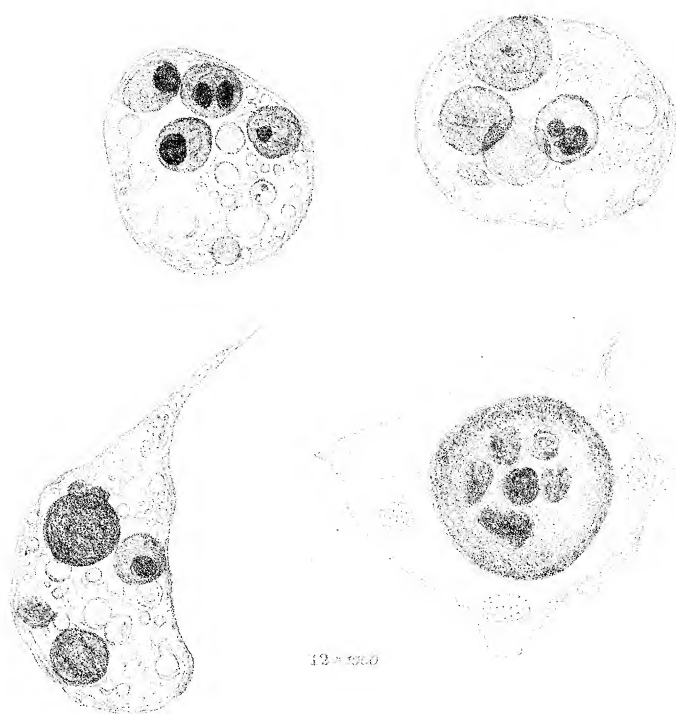
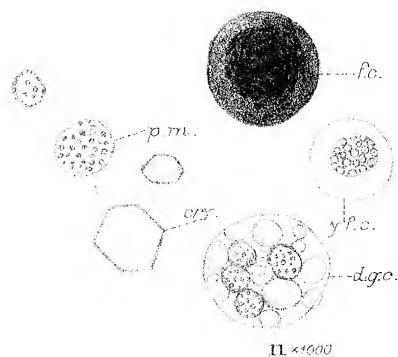
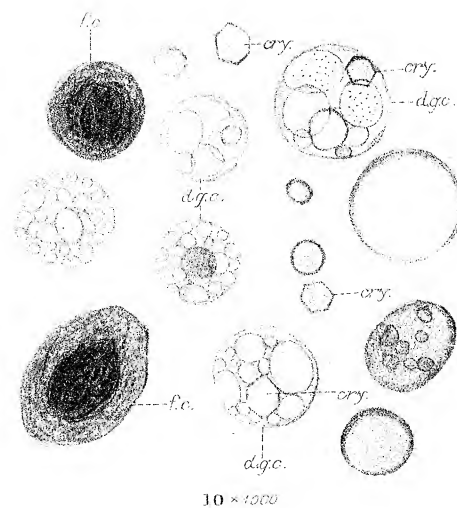
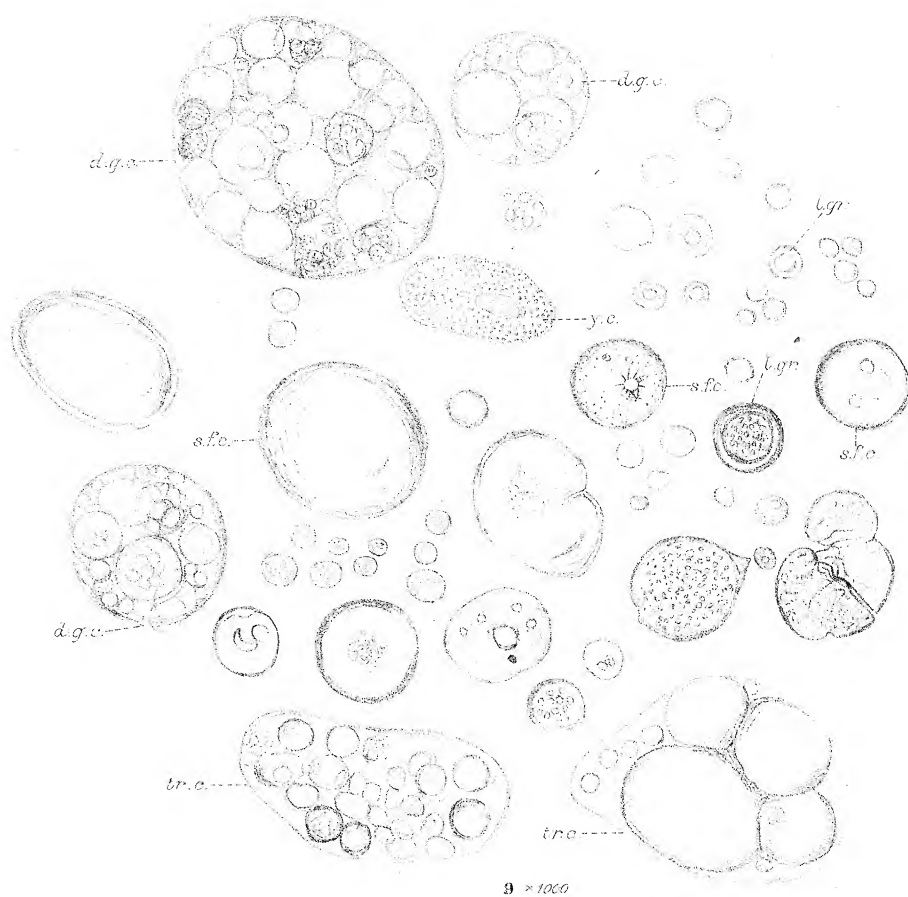
Fig. 15. Longitudinal section of the intestine of *Patella vulgata*, showing absorption of entero-chlorophyll from the intestine, in the lumen of which leucocytes are seen. Within the columnar epithelial cells fine green granules of entero-chlorophyll are seen, while at their base eosinophilous cells, probably leucocytes, are placed.  $\times 240$ . (ZEISS D, ocular 2.) Hæmalum and eosin.

Fig. 16. A portion of the same more highly magnified. The striation of the columnar epithelium (the striated border) and the cilia are well shown. The granules of entero-chlorophyll and the insinuation of the eosinophilous leucocytes between the basal portion of the epithelial cells are well marked.  $\times 1000$ . (ZEISS Apoch. 1/12, comp. ocular 8.) Staining as before.

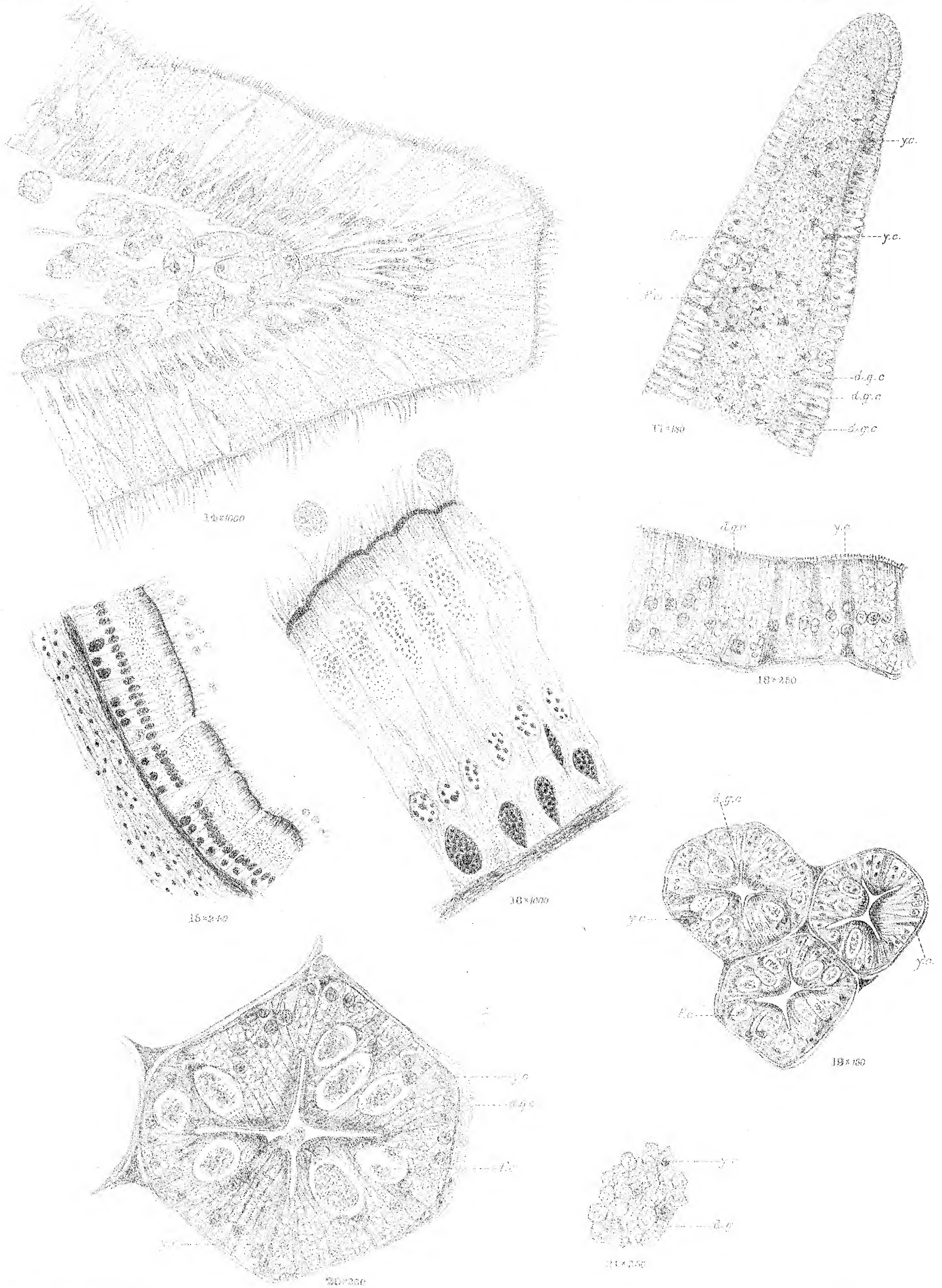
- Fig. 17. An optical section of a cœcal tube of gastric gland of *Homarus vulgaris*, showing the appearance of ferment cells, *f.c.*, fat-holding cells, *d.g.c.*, and young cells, *y.c.* Note the *zonal distribution* of these cells. From a teased preparation, fixed in formol and stained with hæmalum and eosin.  $\times 130$ .
- Fig. 18. Longitudinal section of such a tube showing its wall, in which the same cells are recognisable. Note that some of the young cells reach the lumen of the tube, distinguished by the striated margin. The ciliation not shown.  $\times 250$ .
- Fig. 19. Transverse sections of three tubes, from same gastric gland, showing three kinds of cells, the striation of the cells, and the striated border, but not the cilia.  $\times 130$ .
- Fig. 20. Transverse section of one such tube more highly magnified.  $\times 260$ .
- Fig. 21. Surface view of the fat-holding cells, *d.g.c.*, and young cells, *y.c.*, in a section parallel to the length of a tube.  $\times 250$ .



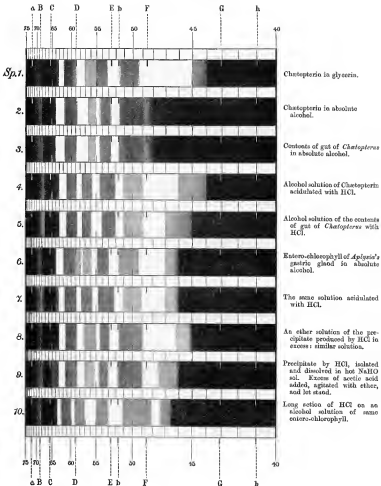


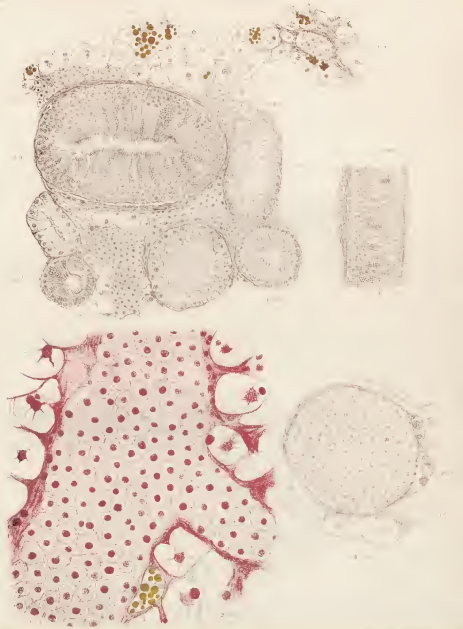






# CHART OF SPECTRA.





# PLATE 1.

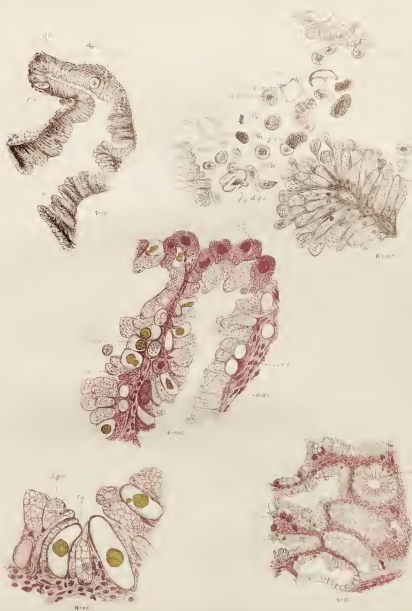
Fig. 1. Section through gastric gland of *Ostrea edulis*, showing in one drawing all the principal parts of the gland; *l.d.*, a large duct showing ciliation, striated border, lining of columnar epithelial cells with eosinophilous granular cells between them; the so-called macroblasts; *s.d.*, small duct; *l.s.d.*, longitudinal section of a large duct; *c.t.*, connective tissue layer or tunica propria; *leuc.*, leucocytes; *v.c.t.*, "vesicular" connective tissue; *pigt. c.*, pigment cells. *b.v.*, blood vessel; *alv.*, section of an alveolus or tube; *macr.*, supposed "macroblasts"; *e.p.c.*, the peculiar epithelioid-celled tissue, seen in fig. 2.  $\times 260$ .

Fig. 2. Longitudinal section of a strand or cylinder of the epithelioid-celled tissue.  $\times 700$ .

Fig. 3. Section of such a strand showing its solid or rod-like character. It is seen surrounded by the vesicular connective tissue of LEYDIG. Two of the cells of the latter containing pigment bodies.  $\times 400$ .

(All stained with hematoxylin and eosin.)





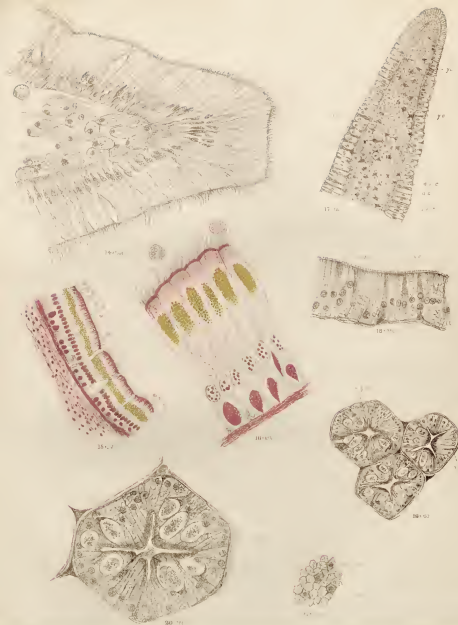
## PLATE 2.

- Fig. 4. Somewhat oblique section through an alveolus of the gastric gland of *Limax*; y.c., young cells; d.g.c., digestive gland cells: equivalent to the hepatic cells of MAX WEBER, or granular cells of FRENZEL; f.c., ferment cells; c.t., connective tissue; c.c., calcareous cells with deeply staining protoplasm.  $\times 250$ .
- Fig. 5. General structure of gland with a low power.  $\times 70$ . Lettering as before.
- Fig. 6. The glandular epithelium under a higher power, showing clear vesicle and coloured sphere in the ferment cells, f.c., and the granular cells, d.g.c.  $\times 450$ .
- Fig. 7. The beginning of a gland duct and its continuation into an alveolus. Lettering as before. The ciliation of the duct is not shown, being hidden by the balsam.  $\times 110$ .
- Fig. 8. This illustrates how all kinds of cells, viz., ferment cells, f.c., granular cells, d.g.c., and calcareous or lime-containing cells c.c. are shed off into the lumen of an alveolus and pass into the intestine. A celloidin preparation.  $\times 260$ .  
(All stained with hæmatoxylin and eosin, and all from *Limax*.)



### PLATE 3.

- Fig. 9. Contents of the mid-gut into which the ducts of the gastric gland open in *Helix hortensis*. In their natural colour. The snail had fasted more than six weeks. All three kinds of cells are represented either as such or by their contents; *d.g.c.*, digestive gland cell or granular cell; *s.f.c.*, spherical or other shaped pigmented mass from the ferment cells. Some very slightly coloured bodies from same source are also present. At the bottom of the figure are two cells, which I take to be transition forms between the granular and ferment cells. *l.gr.*, lime granules from the calcareous cells, which FRENZEL says he never could find in the intestine. Other granules are also present. *y.c.*, a young cell.  $\times 1000$ . (ZEISS Apoch. 1/12, comp. ocular 8.)
- Fig. 10. From the fresh gastric gland of *Aplysia punctata* teased out in sea water. *d.g.c.*, granular cell; *f.c.*, ferment cell contents; *cry.*, crystals.  $\times 1000$ . (ZEISS Apoch. 1/12, comp. ocular 8.)
- Fig. 11. Also from *Aplysia punctata*. Contents of intestine close to gastric gland. *d.g.c.*, granular cell; *y.f.c.*, probably a ferment cell in an early stage; *p.m.*, the pigmented contents from a similar cell; *f.c.*, contents of an adult ferment cell; *cry.*, crystal.  $\times 1000$ . (ZEISS Apoch. 1/12, comp. ocular 8.)
- Fig. 12. The pigmented spheres found in the connective tissue (LEYDIG's vesicular tissue) of *Ostrea edulis*, to be compared with the similar contents of the ferment cells of the gastric gland of other Mollusca. The two upper cells have a resemblance to transition forms, sometimes met with in the glandular epithelium of the gastric gland of other Mollusca.  $\times 1000$ . (ZEISS Apoch. 1/12, comp. ocular 8.)
- Fig. 13. A ferment cell, *f.c.*, with a large vesicle containing small greenish-yellow granules quite similar to some of those in the granular cells, is seen above, and two granular cells below. The protoplasm of *f.c.* is pushed against the periphery of the cell and towards its attached border (to the right). The nucleus occupies the latter part. A young spherical cell with three coloured spheres in its interior occupies the basal protoplasm of the cell. Note the granular character of this protoplasm.  $\times 1000$ . (ZEISS Apoch. 1/12, comp. ocular 8.)



#### PLATE 4.

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